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(71) Applicant *(for all designated States except US)*: LEGOMER PARTNERS, L.P. [US/US]; 50 Oak Avenue, Belmont, MA 02178 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)* : HOGAN, Joseph, C., Jr. [US/US]; 50 Oak Avenue, Belmont, MA 02178 (US).

(74) Agents: CORUZZI, Laura, A. et al.; Pennie & Edmonds, 1155 Avenue of the Americas, New York, NY 10036 (US).

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(57) Abstract

The design and synthesis of novel oxazolone-derived molecular modules and the use of the modules in the construction of new molecules and fabricated materials is disclosed. The new molecules and fabricated materials are molecular recognition agents useful in the design and synthesis of drugs, and have applications in separations and materials science.

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"OXAZOLONE DERIVED MATERIALS".

1. FIELD OF THE INVENTION

The present invention relates to the logical development of biochemical and biopharmaceutical agents and of new materials, including fabricated materials such as fibers, beads, films and gels. Specifically, the invention relates to the development of molecular modules derived from oxazolone (azlactone) and related structures, and to the use of these modules in the assembly of molecules and fabricated materials with tailored properties, which are determined by the contributions of the individual building modules. The molecular modules of the invention are preferably chiral, and can be used to synthesize new compounds and fabricated materials which are able to recognize biological receptors, enzymes, genetic materials, and other chiral molecules, and are thus of great interest in the fields of biopharmaceuticals, separation and materials science.

2. BACKGROUND OF THE INVENTION

The discovery of new molecules has traditionally focused in two broad areas, biologically active molecules, which are used as drugs for the treatment of life-threatening diseases, and new materials, which are used in commercial, especially high-technological applications. In both areas, the strategy used to discover new molecules has involved two basic operations: (i) a more or less random choice of a molecular candidate, prepared either via chemical synthesis or isolated from natural sources, and (ii) the testing of the molecular candidate for the property or properties of interest. This discovery cycle is repeated indefinitely until a molecule possessing the desirable

properties is located. In the majority of cases, the molecular types chosen for testing have belonged to rather narrowly defined chemical classes. For example, the discovery of new peptide hormones has involved work with peptides; the discovery of new therapeutic steroids has involved work with the steroid nucleus; the discovery of new surfaces to be used in the construction of computer chips or sensors has involved work with inorganic materials, etc. As a result, the discovery of new functional molecules, being *ad hoc* in nature and relying predominantly on serendipity, has been an extremely time-consuming, laborious, unpredictable, and costly enterprise.

A brief account of the strategies and tactics used in the discovery of new molecules is described below. The emphasis is on biologically interesting molecules; however, the technical problems encountered in the discovery of biologically active molecules as outlined here are also illustrative of the problems encountered in the discovery of molecules which can serve as new materials for high technological applications. Furthermore, as discussed below, these problems are also illustrative of the problems encountered in the development of fabricated materials for high technological applications.

2.1 Drug Design

Modern theories of biological activity state that biological activities, and therefore physiological states, are the result of molecular recognition events. For example, nucleotides can form complementary base pairs so that complementary single-stranded molecules hybridize resulting in double- or triple-helical structures that appear to be involved in regulation of gene expression. In another example, a biologically active molecule, referr d to as a ligand, binds with

another molecule, usually a macromolecule referred to as ligand-acceptor (e.g. a receptor or an enzyme), and this binding elicits a chain of molecular events which ultimately gives rise to a physiological state, e.g. normal cell growth and differentiation, abnormal cell growth leading to carcinogenesis, blood-pressure regulation, nerve-impulse generation and propagation, etc. The binding between ligand and ligand-acceptor is geometrically characteristic and extraordinarily specific, involving appropriate three-dimensional structural arrangements and chemical interactions.

2.1.1 Design and Synthesis of Nucleotides

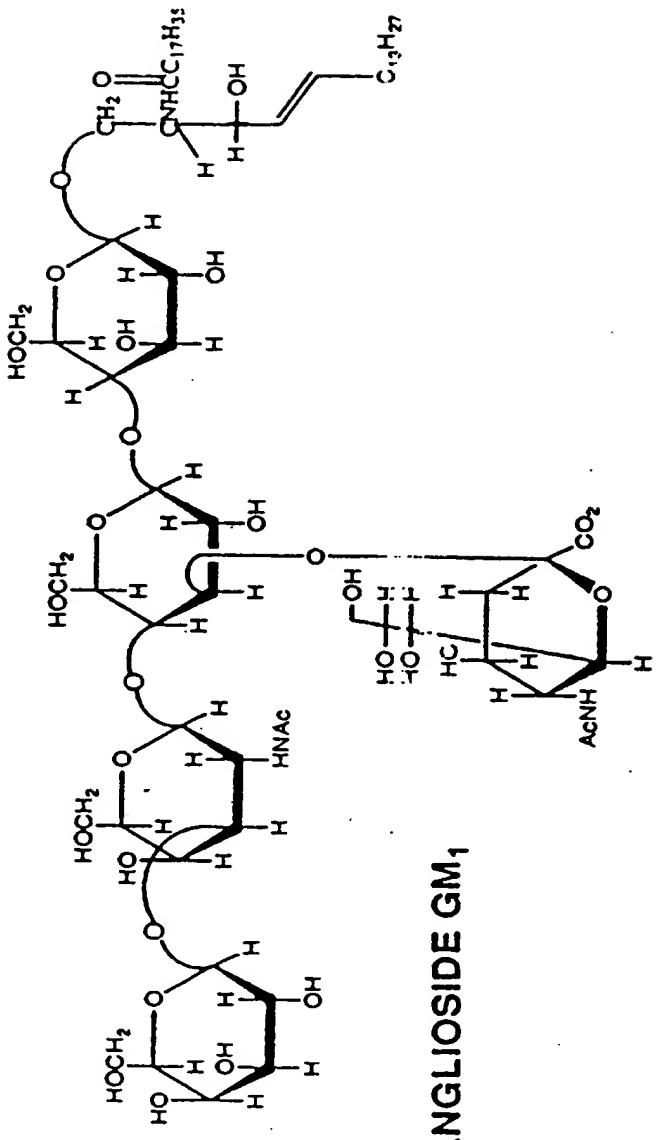
Recent interest in gene therapy and the manipulation of gene expression has focused on the design of synthetic oligonucleotides that can be used to block or suppress gene expression via an antisense, ribozyme or triple helix mechanism. To this end, the sequence of the native target DNA or RNA molecule is characterized and standard methods are used to synthesize oligonucleotides representing the complement of the desired target sequence (see, S. Crooke, The FASEB Journal, Vol. 7, Apr. 1993, p. 533 and references cited therein). Attempts to design more stable forms of such oligonucleotides for use *in vivo* have typically involved the addition of various functional groups, e.g., halogens, azido, nitro, methyl, keto, etc. to various positions of the ribose or deoxyribose subunits cf., The Organic Chemistry of Nucleic Acids, Y. Mizuno, Elsevier Science Publishers BV, Amsterdam, The Netherlands, 1987.

2.1.2 GLYCOPEPTIDES

As a result of recent advances in biological carbohydrate chemistry, carbohydrates are being increasingly viewed as the components of living systems with the enormously complex structures required for the

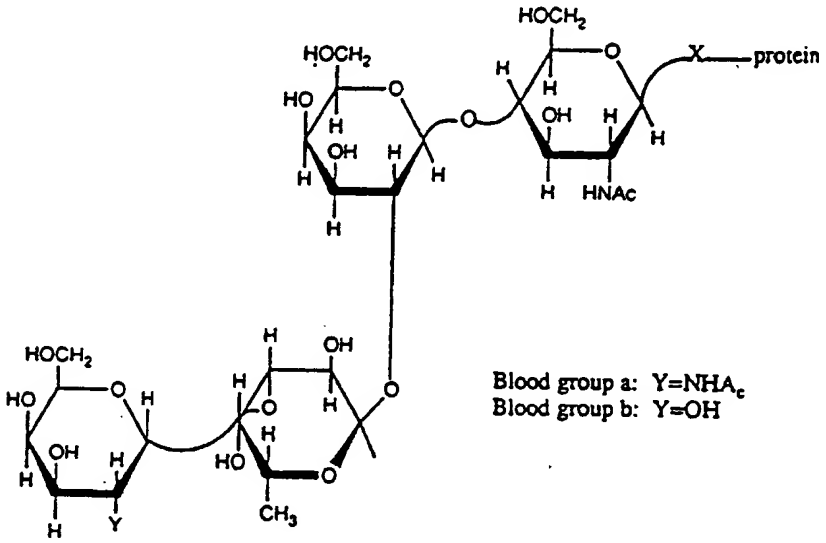
encoding of the massive amounts of information needed to orchestrate the processes of life, *e.g.*, cellular recognition, immunity, embryonic development, carcinogenesis and cell-death. Thus, whereas two naturally occurring amino acids can be used by nature to convey 2 fundamental molecular messages, *i.e.*, via formation of the two possible dipeptide structures, and four different nucleotides convey 24 molecular messages, two different monosaccharide subunits can give rise to 11 unique disaccharides, and four dissimilar monosaccharides can give rise to up to 35,560 unique tetramers each capable of functioning as a fundamental molecular message in a given physiological system.

The gangliosides are examples of the versatility and effect with which organisms can use saccharide structures. These molecules are glycolipids (sugar-lipid composites) and as such are able to position themselves at strategic locations on the cell wall: their lipid component enables them to anchor in the hydropholic interior of the cell wall, positioning their hydrophilic component in the aqueous extracellular *millieu*. Thus the gangliosides (like many other saccharides) have been chosen to act as cellular sentries: they are involved in both the inactivation of bacterial toxins and in *contact inhibition*, the latter being the complex and poorly understood process by which normal cells inhibit the growth of adjacent cells, a property lost in most tumor cells. The structure of ganglioside GM, a potent inhibitor of the toxin secreted by the cholera organism, featuring a branched complex pentameric structure is shown below.

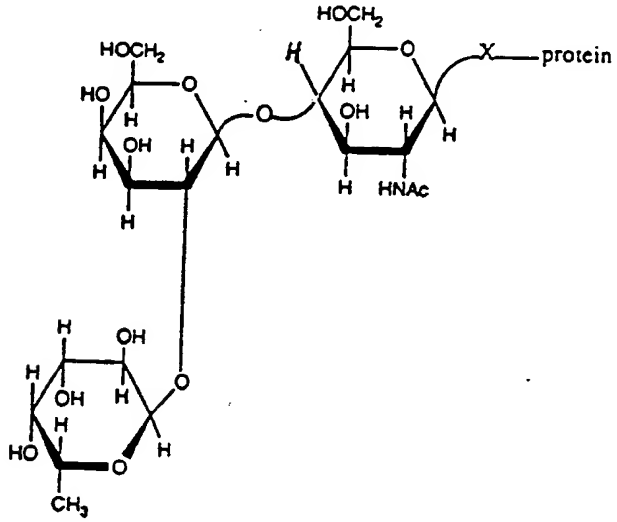


GANGLIOSIDE GM_1

The oligosaccharide components of the glycoproteins (sugar-protein composites) responsible for the human blood-group antigens (the A, B, and O blood classes) are shown below.



BLOOD GROUP A AND B ANTIGENS



BLOOD GROUP O ANTIGEN, TYPE II

Interactions involving complementary proteins and glycoproteins on red blood cells belonging to incompatible blood classes cause formation of aggregates, or clusters and are the cause for failed transfusions of human blood.

Numerous other biological processes and macromolecules are controlled by glycosylation (*i.e.*, the covalent linking with sugars). Thus, glycosylation of erythropoetin causes loss of the hormone's biological activity; deglycosylation of human gonadotropic hormone increases receptor binding but results in almost complete loss of biological activity (see Rademacher et al., Ann. Rev. Biochem 57, 785 (1988); and glycosylation of three sites in tissue plasminogen activating factor (TPA) produces a glycopolypeptide which is 30% more active than the polypeptide that has been glycosylated at two of the sites.

2.1.3 Design and Synthesis of Mimetics of Biological Ligands

A currently favored strategy for development of agents which can be used to treat diseases involves the discovery of forms of ligands of biological receptors, enzymes, or related macromolecules, which mimic such ligands and either boost, i.e., agonize, or suppress, i.e., antagonize, the activity of the ligand. The discovery of such desirable ligand forms has traditionally been carried out either by random screening of molecules (produced through chemical synthesis or isolated from natural sources), or by using a so-called "rational" approach involving identification of a lead-structure, usually the structure of the native ligand, and optimization of its properties through numerous cycles of structural redesign and biological testing. Since most useful drugs have been discovered not through the "rational" approach but through the screening of randomly chosen compounds, a hybrid approach to drug discovery has recently emerged which is based on the use of combinatorial chemistry to construct huge libraries of randomly-built chemical structures which are screened for specific biological activities. (S. Brenner and R.A. Lerner, 1992, Proc. Natl. Acad. Sci. USA 89:5381)

Most lead-structures which have been used in "rational" drug design are native polypeptide ligands of receptors or enzymes. The majority of polypeptide ligands, especially the small ones, are relatively unstable in physiological fluids due to the tendency of the peptide bond to undergo facile hydrolysis in acidic media or in the presence of peptidases. Thus, such ligands are decisively inferior in a pharmacokinetic sense to nonpeptidic compounds, and are not favored as drugs. An additional limitation of small peptides as drugs is their low affinity for ligand acceptors. This phenomenon is in sharp contrast to the affinity demonstrated by large, folded polypeptides, e.g. proteins, for specific acceptors, e.g. receptors or

enzymes, which is in th subnanomolar range. For peptides to become effective drugs, they must be transformed into nonpeptidic organic structures, i.e., peptide mimetics, which bind tightly, preferably in the nanomolar range, and can withstand the chemical and biochemical rigors of coexistence with biological fluids.

Despite numerous incremental advances in the art of peptidomimetic design, no general solution to the problem of converting a polypeptide-ligand structure to a peptidomimetic has been defined. At present, "rational" peptidomimetic design is done on an *ad hoc* basis. Using numerous redesign-synthesis-screening cycles, peptidic ligands belonging to a certain biochemical class have been converted by groups of organic chemists and pharmacologists to specific peptidomimetics; however, in the majority of cases the results in one biochemical area, e.g. peptidase inhibitor design using the enzyme substrate as a lead, cannot be transferred for use in another area, e.g. tyrosine-kinase inhibitor design using the kinase substrate as a lead.

In many cases, the peptidomimetics that result from a peptide structural lead using the "rational" approach comprise unnatural α-amino acids. Many of these mimetics exhibit several of the troublesome features of native peptides (which also comprise α-amino acids) and are, thus, not favored for use as drugs. Recently, fundamental research on the use of nonpeptidic scaffolds, such as steroidal or sugar structures, to anchor specific receptor-binding groups in fixed geometric relationships have been described (see for example Hirschmann, R. et al., 1992 J. Am. Chem. Soc., 114:9699-9701; Hirschmann, R. et al., 1992 J. Am. Chem. Soc., 114:9217-9218); however, the success of this approach remains to be seen.

In an attempt to accelerate the identification of lead-structures, and also the identification of useful drug candidates through screening of randomly chosen

c mpounds, researchers have developed automated methods for the generation of large combinatorial libraries of peptides and certain types of peptide mimetics, called "peptoids", which are screened for a desirable biological activity. For example, the method of H. M. Geysen, (1984 Proc. Natl. Acad. Sci. USA 81:3998) employs a modification of Merrifield peptide synthesis wherein the C-terminal amino acid residues of the peptides to be synthesized are linked to solid-support particles shaped as polyethylene pins; these pins are treated individually or collectively in sequence to introduce additional amino-acid residues forming the desired peptides. The peptides are then screened for activity without removing them from the pins. Houghton, (1985, Proc. Natl. Acad. Sci. USA 82:5131; and U.S. Patent No. 4,631,211) utilizes individual polyethylene bags ("tea bags") containing C-terminal amino acids bound to a solid support. These are mixed and coupled with the requisite amino acids using solid phase synthesis techniques. The peptides produced are then recovered and tested individually. Fodor et al., (1991, Science 251:767) described light-directed, spatially addressable parallel-peptide synthesis on a silicon wafer to generate large arrays of addressable peptides that can be directly tested for binding to biological targets. These workers have also developed recombinant DNA/genetic engineering methods for expressing huge peptide libraries on the surface of phages (Cwirla et al., 1990, Proc. Natl. Acad. Sci. USA 87:6378).

In another combinatorial approach, V. D. Huebner and D.V. Santi (U.S. Patent No. 5,182,366) utilized functionalized polystyrene beads divided into portions each of which was acylated with a desired amino acid; the bead portions were mixed together and then split into portions each of which was subjected to acylation with a second desirable amino acid producing

dipeptides, using the techniques of solid phase peptide synthesis. By using this synthetic scheme, exponentially increasing numbers of peptides were produced in uniform amounts which were then separately screened for a biological activity of interest.

Zuckerman et al., (1992, Int. J. Peptide Protein Res. 91:1) also have developed similar methods for the synthesis of peptide libraries and applied these methods to the automation of a modular synthetic chemistry for the production of libraries of N-alkyl glycine peptide derivatives, called "peptoids", which are screened for activity against a variety of biochemical targets. (See also, Symon et al., 1992, Proc. Natl. Acad. Sci. USA 89:9367). Encoded combinatorial chemical syntheses have been described recently (S. Brenner and R.A. Lerner, 1992, Proc. Natl. Acad. Sci. USA 89:5381).

In addition to the lead structure, a very useful source of information for the realization of the preferred "rational" drug discovery is the structure of the biological ligand acceptor which, often in conjunction with molecular modelling calculations, is used to simulate modes of binding of the ligand with its acceptor; information on the mode of binding is useful in optimizing the binding properties of the lead-structure. However, finding the structure of the ligand acceptor, or preferably the structure of a complex of the acceptor with a high affinity ligand, requires the isolation of the acceptor or complex in the pure, crystalline state, followed by x-ray crystallographic analysis. The isolation and purification of biological receptors, enzymes, and the polypeptide substrates thereof are time-consuming, laborious, and expensive; success in this important area of biological chemistry depends on the effective utilization of sophisticated separation technologies.

Crystallization can be valuable as a separation technique but in the majority of cases, especially in cases involving isolation of a biomolecule from a complex biological milieu, successful separation is chromatographic. Chromatographic separations are the result of reversible differential binding of the components of a mixture as the mixture moves on an active natural, synthetic, or semisynthetic surface; tight-binding components in the moving mixture leave the surface last *en masse* resulting in separation.

The development of substrates or supports to be used in separations has involved either the polymerization crosslinking of monomeric molecules under various conditions to produce fabricated materials such as beads, gels, or films, or the chemical modification of various commercially available fabricated materials, e.g., sulfonation of polystyrene beads, to produce the desired new materials. Prior art support materials have been developed to perform specific separations or types of separations and are of limited utility. Many of these materials are incompatible with biological macromolecules, e.g. reverse-phase silica frequently used to perform high pressure liquid chromatography can denature hydrophobic proteins and other polypeptides. Furthermore, many supports are used under conditions which are not compatible with sensitive biomolecules, such as proteins, enzymes, glycoproteins, etc., which are readily denaturable and sensitive to extreme pH's. An additional difficulty with separations carried out using these supports is that the separation results are often support-batch dependent, i.e., they are irreproducible.

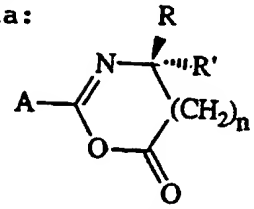
Recently a variety of coatings and composite-forming materials have been used to modify commercially available fabricated materials into articles with improved properties; however the success of this approach remains to be seen.

If a chromatographic surface is equipped with molecules which bind specifically with a component of a complex mixture, that component will be separated from the mixture and may subsequently be released by changing the experimental conditions, (e.g. buffers, stringency, etc.) This type of separation is appropriately called affinity chromatography and remains an extremely effective and widely used separation technique. It is certainly much more selective than traditional chromatographic techniques, e.g chromatography on silica, alumina, silica or alumina coated with long-chain hydrocarbons, polysaccharide and other types of beads or gels, etc., which in order to attain their maximum separating efficiency need to be used under conditions that are damaging to biomolecules, e.g. conditions involving high pressure, use of organic solvents and other denaturing agents, etc.

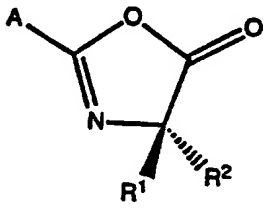
The development of more powerful separation technologies depends significantly on breakthroughs in the field of materials science, specifically in the design and construction of materials that have the power to recognize specific molecular shapes under experimental conditions resembling those found in physiological media, i.e. these experimental conditions must involve an aqueous medium whose temperature and pH are close to the physiological levels and which contains none of the agents known to damage or denature biomolecules. The construction of these "intelligent" materials frequently involves the introduction of small molecules capable of specifically recognizing others into existing materials, e.g. surfaces, films, gels, beads, etc., by a wide variety of chemical modifications. Alternatively, molecules capable of recognition are converted to monomers and used to create the "intelligent" materials through polymerization r actions.

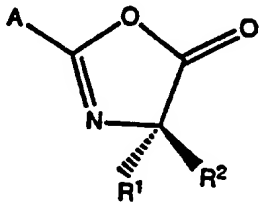
2.2 Oxazolones

Oxazolones, or azlactones, are structures of the general formula:



where A is a functional group and n is 0 or 1 and typically 1-3. Oxazolones containing a five-membered ring and a single substituent at position 4 are typically encountered as transient intermediates which cause problematic racemization during the chemical synthesis of peptides. An oxazolone can in principle contain one or two substituents at the 4-position. When these substituents are not equivalent, the carbon atom at the 4-position is asymmetric and two non-superimposable oxazolone structures (azlactones) result:

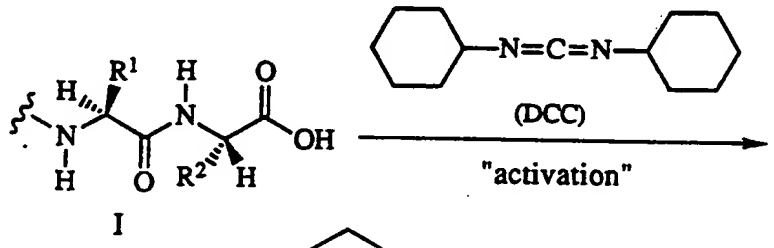




Chiral oxazolones possessing a single 4-substituent (also known as 5(4H)-oxazolones), derived from (chiral) natural amino acid derivatives, including activated acylamino acyl structures, have been prepared and isolated in the pure, crystalline state (Bodansky, M.; Klausner, Y.S.; Ondetti, M.A. in "Peptide Synthesis", Second Edition, John Wiley & Sons, New York, 1976, p. 14 and references cited therein). The facile, base-catalyzed racemization of several of these oxazolones has been studied in connection with investigations of the serious racemization problem confronting peptide synthesis (see K mp, D.S. in "The Peptides, Analysis,

Synthesis, and Biology", Vol. 1, Gross, E. & Meienhofer, J. editors, 1979, p. 315).

Racemization during peptide synthesis becomes very extensive when the desired peptide is produced by aminolysis of activated peptidyl carboxyl, as in the case of peptide chain extension from the amino terminus, e.g. I → VI shown below (see Atherton, E.; Sheppard, R.C. "Solid Phase Peptide Synthesis, A Practical Approach", IRL Press at Oxford University Press, 1989, pages 11 and 12). An extensively studied mechanism describing this racemization involves conversion of the activated acyl derivative (II) to an oxazolone (III) followed by facile base-catalyzed racemization of the oxazolone via a resonance-stabilized intermediate (IV) and aminolysis of the racemic oxazolone (V) producing racemic peptide products (VI).



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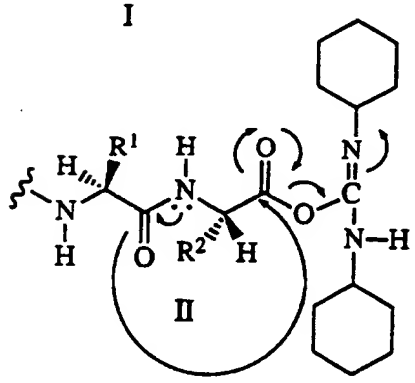
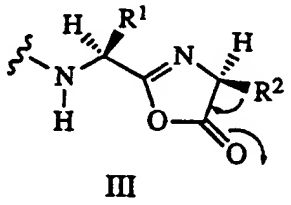
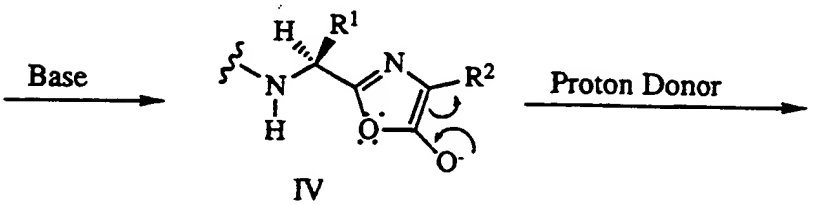


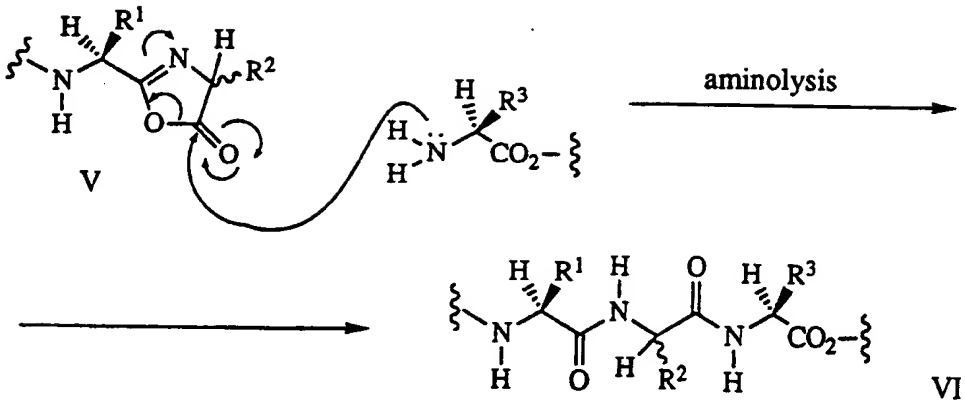
II



III



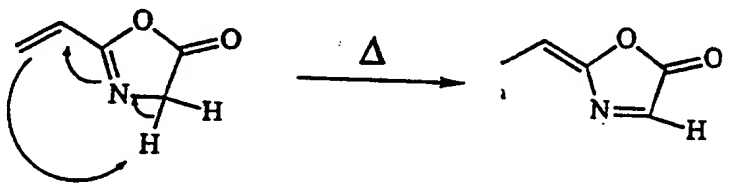
IV



Extensive research on the trapping of oxazolones III (or of their activated acyl precursors II) to give acylating agents which undergo little or no racemization upon aminolysis has been carried out, and successes in this area (such as the use of N-hydroxybenzotriazole) have greatly advanced the art of peptide synthesis (Kemp, D.S. in "The Peptides, Analysis, Synthesis, and Biology", Vol. 1, Gross, E. & Meienhofer, J. editors, 1979, p. 315).

Thus, attempts to deal with the racemization problem in peptide synthesis have involved suppressing or avoiding the formation of oxazolone intermediates altogether.

Furthermore, certain vinyl oxazolones having a hydrogen substituent at the 4-position can also undergo thermal rearrangements (23 Tetrahedron 3363 (1967)), which may interfere with other desired transformations, such as Michael-type additions.



3. SUMMARY OF THE INVENTION

A new approach to the construction of novel molecules is described. This approach involves the development of oxazolone (azlactone) derivative molecular building blocks, containing appropriate atoms and functional groups which may be chiral and which are used in a modular assembly of molecules with tailored properties; each module contributing to the overall properties of the assembled molecule. The oxazolone derivative building blocks of the invention can be used to synthesize novel molecules designed to mimic the three-dimensional structure and function of native ligands, and/or interact with the binding sites of a native receptor. This logical approach to molecular construction is applicable to the synthesis of all types of molecules, including but not limited to mimetics of peptides, proteins, oligonucleotides, carbohydrates, lipids, polymers and to fabricated materials useful in materials science. It is analogous to the modular construction of a mechanical device that performs a specific operation wherein each module performs a specific task contributing to the overall operation of the device.

The invention is based, in part, on the following insights of the discoverer. (1) All ligands share a single universal architectural feature: they consist of a scaffold structure, made e.g. of amide, carbon-carbon, or phosphodiester bonds which support several functional groups in a precise and relatively rigid geometric arrangement. (2) Binding modes between ligands and receptors share a single universal feature as well: they all involve attractive interactions between complementary structural elements, e.g., charge- and π-type interactions, hydrophobic and van der Waals forces, hydrogen bonds. (3) A continuum of fabricated materials exists spanning a dimensional range from about 100 Å to

1 cm in diameter comprising various materials of construction, geometries, morphologies, and functions, all possessing the common feature of a functional surface which is presented to a biologically active molecule or a mixture of molecules to achieve recognition between the molecule (or the desired molecule in a mixture) and the surface. And (4) Oxazolone derivative structures, heretofore regarded as unwanted intermediates which form during the synthesis of peptides, would be ideal building blocks for constructing backbones or scaffolds bearing the appropriate functional groups that either mimic desired ligands, and/or interact with appropriate receptor binding sites, and for carrying out the synthesis of the various parts of the functionalized scaffold orthogonally, provided that racemization of the oxazolone structures is prevented or controlled. Thus, the invention is also based, in part, on the further recognition that such derivatives of ozaxolones, which do not racemize, can be used as universal building blocks for the synthesis of such novel molecules. Furthermore, oxazolone derivatives may be utilized in a variety of ways across the continuum of fabricated materials described above to produce new materials capable of specific molecular recognition. These oxazolone derivatives may be chirally pure and used to synthesize molecules that mimic a number of biologically active molecules, including but not limited to peptides, proteins, oligonucleotides, polynucleotides, carbohydrates and lipids, and a variety of other polymers as well as fabricated materials that are useful as new materials, including but not limited to solid supports useful in column chromatography, catalysts, solid phase immunoassays, drug delivery vehicles, films, and "intelligent" materials designed for use in selective separations of various components of complex mixtures.

Working examples describing the use of oxazolone-derived modules in the modular assembly of a variety of molecular structures are given. The molecular structures include functionalized silica surfaces useful in the optical resolution of racemic mixtures; peptide mimetics which inhibit human elastase, protein-kinase, and the HIV protease; and polymers formed via free-radical or condensation polymerization of oxazolone-containing monomers.

In accordance with the present invention, the oxazolone-derived molecules of interest possess the desired stereochemistry and, when required, are obtained enantiomerically pure. In addition to the synthesis of single molecular entities, the synthesis of libraries of oxazolone-derived molecules, using the techniques described herein or modifications thereof which are well known in the art to perform combinatorial chemistry, is also within the scope of the invention. Furthermore, the oxazolone-derived molecules possess enhanced hydrolytic and enzymatic stabilities, and in the case of biologically active materials, are transported to target ligand-acceptor macromolecules *in vivo*, without causing any serious side-effects.

According to the present invention, chiral oxazolones, in which the asymmetric center is a 4-disubstituted carbon, as well as synthetic nonchiral oxazolones may be synthesized readily and used as molecular modules capable of controlled reaction with a variety of other molecules to produce designed chiral recognition agents and conjugates. These chiral oxazolones may also be linked together, using polymerizing reactions carried out either in a stepwise or chain manner, to produce polymeric biological ligand mimics of defined sequence and stereochemistry. Furthermore, according to the present invention,

4-disubstituted chiral oxazolones are extremely useful in the asymmetric functionalization of various solid supports and biological macromolecules and in the production of various chiral polymers with useful properties. The products of all of these reactions are surprisingly stable in diverse chemical and enzymological environments, and uniquely suitable for a variety of superior pharmaceutical and high-technological applications.

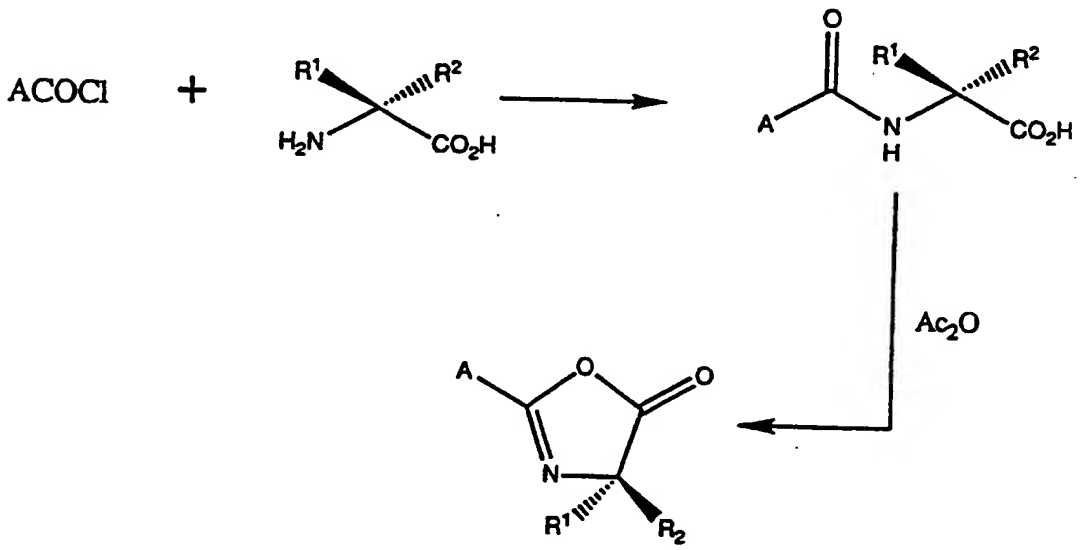
For applications in which the 4 position of the oxazolone precursor does not need to be chiral, e.g., the construction of certain polymeric materials, the use of oxazolones in the construction of linkers for the joining of two or more pharmaceutically useful or, simply, biologically active ligands, etc., symmetric or nonchiral oxazolones are used in chemical syntheses. Furthermore, if the oxazolone-derived product does not need to incorporate the 4-position of the oxazolone precursor in the enantiomerically pure state, oxazolone precursors which are not enantiomerically pure may be used for syntheses.

4. DETAILED DESCRIPTION OF THE INVENTION

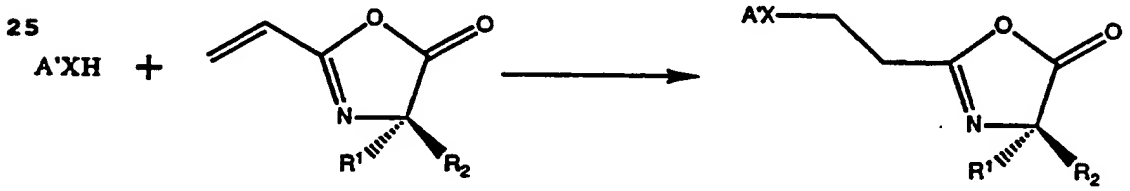
To the extent necessary to further understand any portion of the detailed description, the following earlier filed U.S. patent applications are expressly incorporated herein by reference thereto: DISUBSTITUTED OXAZOLONE COMPOSITIONS AND DERIVATIVES THEREOF (Serial No. 07/906,756 filed June 30, 1992); and DIRECTED CHIRAL LIGANDS, RECOGNITION AGENTS AND FUNCTIONALLY USEFUL MATERIALS FROM SUBSTITUTED OXAZOLONES AND DERIVATIVES CONTAINING AN ASYMMETRIC CENTER (Serial No. 08/041,562 filed April 2, 1993).

4.1 Synthesis of Chiral Substituted Oxazolones

Chiral 4,4'-disubstituted oxazolones may be prepared from the appropriate N-acyl amino acid using any of a number of standard acylation and cyclization techniques well-known to those skilled in the art, e.g.:



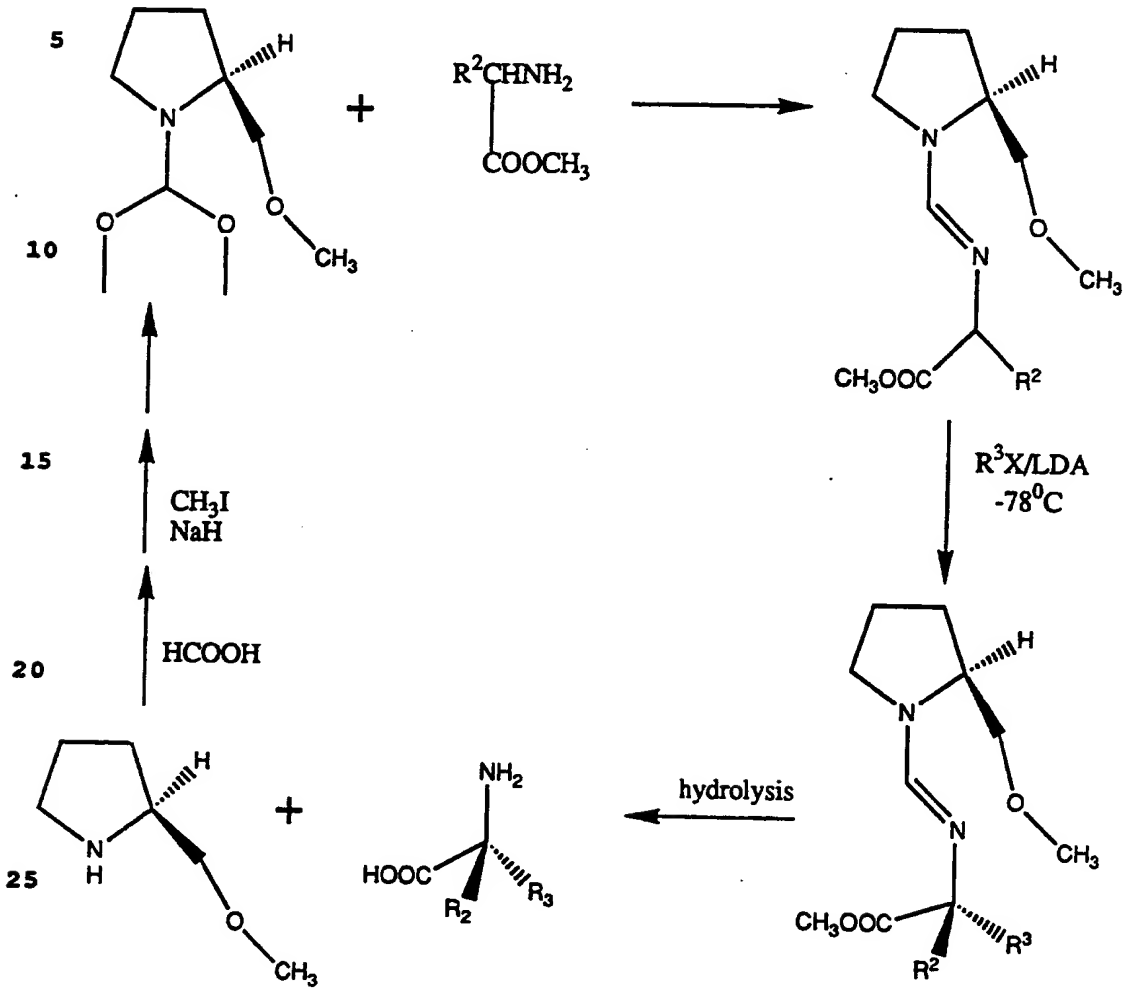
If the substituent at the 2-position is capable of undergoing addition reactions, these may be carried out with retention of the chirality at the 4-position to produce new oxazolones. This is shown for the Michael addition to an alkenyl oxazolone as follows:



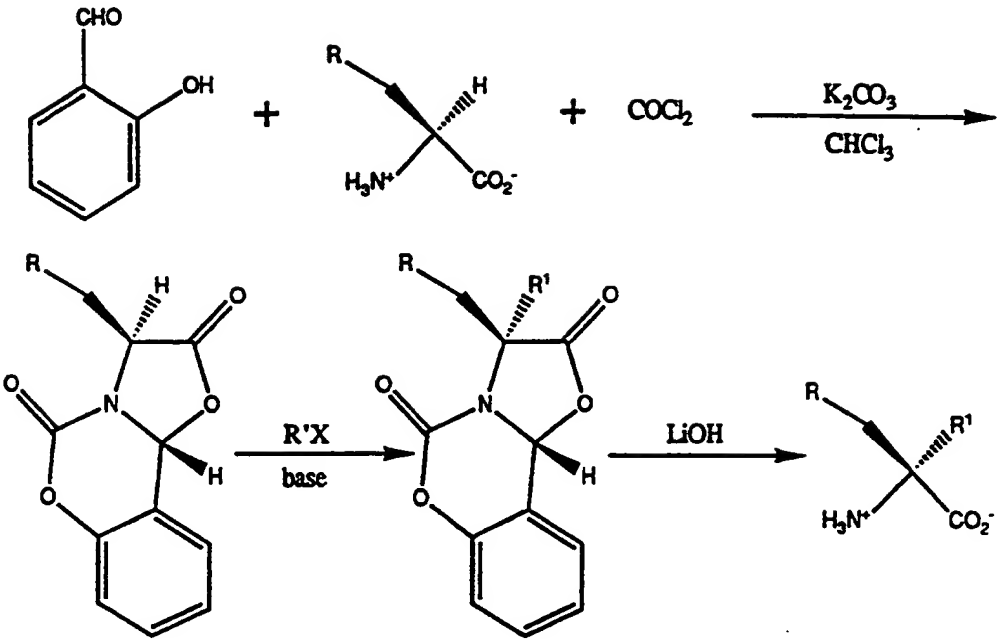
where X = S or NR and A' is a functionalized alkyl group.

The required chiral amino acid precursors for oxazolone synthesis may be produced using stereoselective reactions that employ chiral auxiliaries. An example of such a chiral auxiliary is (5)-(-)-1-dimethoxymethyl-2-

methoxymethylpyrrolidine (SMPD) (Liebig's Ann. Chem. 1668 (1983)) as shown below,

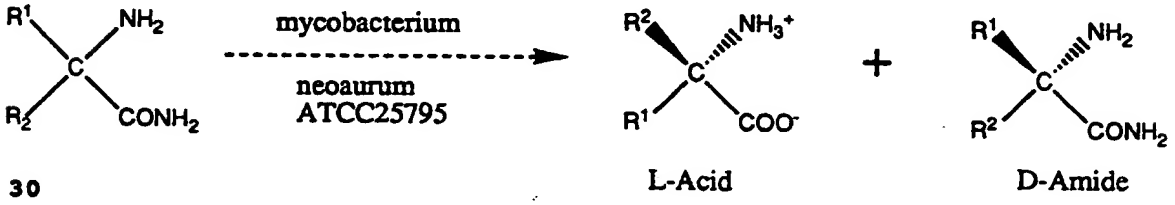


wherein R^2 = CH_3, *i*-Bu, or benzyl; and R^3 = CH_3, CHF_2, C_2H_5, n-Bu, or benzyl. A second example involves 5H,10bH-oxazolo[3,2-c][1,3]benzoxazine-2(3H),5-diones (55 J. Org. Chem. 5437 (1990)),



wherein R^1 = phenyl or i-Pr; and R^2 = CH_3, C_2H_5, or CH_2=CH-CH_2.

Alternatively, the desired chiral amino acid may be obtained using stereoselective biochemical transformations carried out on the racemate, synthesized via standard reactions, as shown below for a case involving a commercially-available organism (53 J. Org. Chem. 1826 (1988)),

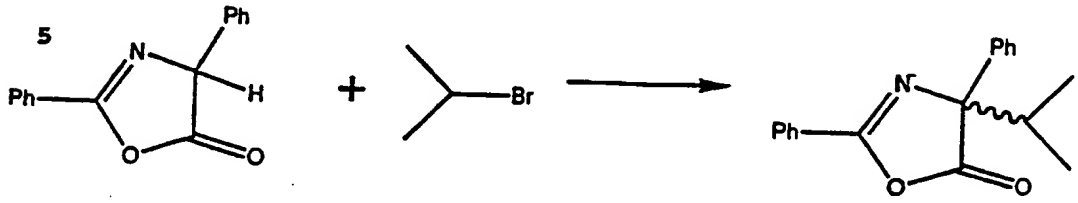


L-Acid D-Amide

wherein R^1 = i-Pr, i-Bu, phenyl, benzyl, p-methoxybenzyl, or phenethyl; and R = CH_3 or C_2H_5.

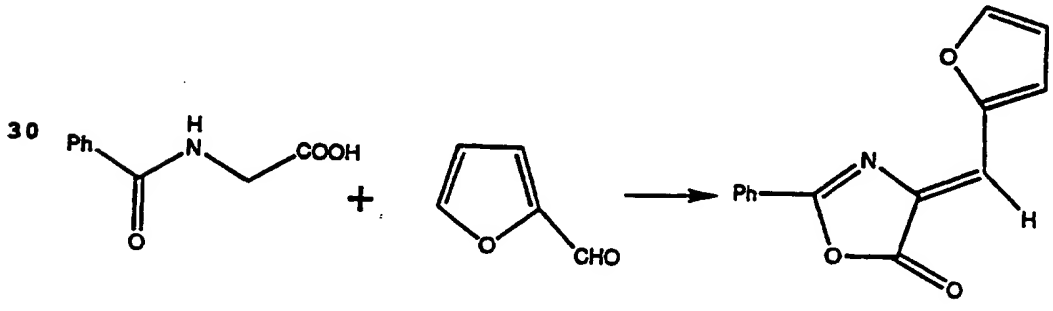
Racemic mixtures of 4,4'-disubstituted xazolones may be prepared from monosubstituted

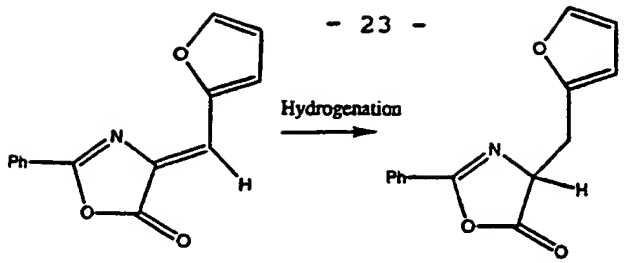
oxazolones by alkylation of the 4-position, as in the following transformation (Synthesis Commun., Sept. 1984, at 763; 23 Tetrahedron Lett. 4259 (1982)):



Resolution of racemic mixtures of oxalolones may be effected using chromatography or chiral supports under suitable conditions which are well known in the art; using fractional crystallization of stable salts of oxazolones with chiral acids; or simply by hydrolyzizing the racemic oxazolone to the amino acid derivative and resolving the racemic modification using standard analytical techniques.

A wide variety of 4-monosubstituted azlactones may be readily prepared by reduction of the corresponding unsaturated derivatives obtained in high yield from the condensation reaction of aldehydes, ketones, or imines with the oxazolone formed from an N-acyl glycine (49 J. Org. Chem. 2502 (1984); 418 Synthesis Communications (1984))



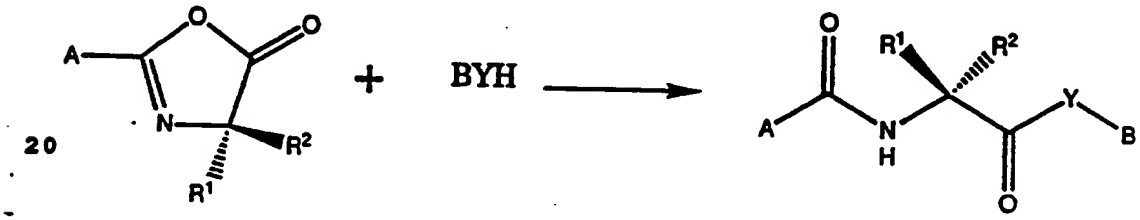


Thus, the art provides a wealth of chemical and biochemical methods which can be used to produce a wide variety of enantiomeric, multifunctionalized oxazolones whose substituents may be tailored to mimic any desirable form of the side chains of native polypeptides.

4.2 Synthetic Transformations of Chiral Oxazolones

4.2.1 Reactions with One or Two Nucleophiles Producing Conjugates

Chiral oxazolones may be subjected to ring-opening reactions with a variety of nucleophiles producing chiral molecules as shown below:



In the structure above, Y represents an oxygen, sulfur, or nitrogen atom. R^1 and R^2 differ from one another and taken alone each signifies one of the followng: alkyl including cycloalkyl and substituted forms thereof; aryl, aralkyl, alkaryl, and substituted or heterocyclic versions thereof; preferred forms of R^1 and R^2 are structures mimicking the side chains of naturally-occurring amino acids as well as various ring structures.

The above ring-opening reaction can be carried out either in an organic solvent such as methylene chloride, ethyl acetate, dimethyl formamide (DMF) or in water at room or higher temperatures, in the presence or

absence of acids, such as carboxylic, other proton or Lewis-acids, or bases, such as tertiary amines or hydroxides, serving as catalysts. If structure BYH contains nucleophilic functional groups which may interfere with the ring-opening acylation, these groups must be temporarily protected using suitable orthogonal protection strategies based on the many protecting groups known in the art; cf., e.g., Protective Groups in Organic Synthesis, 2ed., T.W. Greene and P.G.M. Wuts, John Wiley & Sons, New York, N.Y., 1991.

The substituents A and B shown may be of a variety of structures and may differ markedly in their physical or functional properties, or may be the same; they may also be chiral or symmetric. A and B are preferably selected from:

1) an amino acid derivative of the form $(AA)_n$, which would include natural and synthetic amino acid residues (n=1), peptides (n=2-30), polypeptides (n=31-70) and proteins (n>70). These derivatives are generally connected to the amine of the amino acyl structure used to form the oxazolone through a carbonyl group, although other reactions which are known to functionalize terminal amino groups may be employed. It is recognized that certain amino acid derivatives would already contain the necessary connecting group, such as a carbonyl, so that a direct chemical bond can be obtained to the product of the oxazolone ring opening reaction without the use of a connecting group.

2) a nucleotide derivative of the form $(NUCL)_n$, which would include natural and synthetic nucleotides (n=1), nucleotide probes (n=2-25) and oligonucleotides (n>25) including both deoxyribose (DNA) and ribose (RNA) variants.

3) a carbohydrate derivative of the form $(CH)_n$. This would include natural physiologically active carbohydrates (glucose, galactose, etc.) including related compounds such as sialic acids, etc. (n=1), synthetic carbohydrate residues and derivatives of these (n=1) and all of the complex oligomeric permutations of these as found in nature (n>1) cf. Scientific American, January 1993, p. 82.

4) a naturally occurring or synthetic organic structural motif. This term includes any of the well known base structures of pharmaceutical compounds including pharmacophores or metabolites thereof. These structural motifs are generally known to have specific desirable binding properties to ligand acceptors of interest and would include structures other than those recited above in 1), 2) and 3).

5) a reporter element such as a natural or synthetic dye or a residue capable of photographic amplification which possesses reactive groups which may be synthetically incorporated into the oxazolone structure or reaction scheme and may be attached through the groups without adversely interfering with the reporting functionality of the group. Preferred reactive groups are amino, thio, hydroxy, carboxylic acid, acid chloride, isocyanate alkyl halides, aryl halides and oxirane groups.

6) an organic moiety containing a polymerizable group such as a double bond or other functionalities capable of undergoing condensation polymerization or copolym rization. Suitable groups include

vinyl groups, oxirane groups, carboxylic acids, acid chlorides, esters, amides, lactones and lactams.

7) a macromolecular component, such as a macromolecular surface or structures which may be attached to the oxazolone modules via the various reactive groups outlined above in a manner where the binding of the attached species to a ligand-receptor molecule is not adversely affected and the interactive activity of the attached functionality is determined or limited by the macromolecule. The molecular weight of these macromolecules may range from about 1000 Daltons to as high as possible. They may take the form of nanoparticles (d_p=100-1000Å), latex particles (d_p=1000Å-5000Å), porous or non-porous beads (d_p=0.5μ-1000μ), membranes, gels, macroscopic surfaces or functionalized or coated versions or composites of these.

Under certain circumstances, A and/or B may be a chemical bond to a suitable organic moiety, a hydrogen atom, an organic moiety which contains a suitable electrophilic group, such as an aldehyde, ester, alkyl halide, ketone, nitrile, epoxide or the like, a suitable nucleophilic group, such as a hydroxyl, amino, carboxylate, aminde, carbanion, urea or the like, or one of the R groups defined below. In addition, A and B may join to form a ring or structure which connects to the ends of the repeating unit of the compound defined by the preceding formula or may be separately connected to other moeities.

A more generalized presentation of the composition of the invention is defined by the structure

wherein:

a. at least one of A and B are as defined above and A and B are optionally connected to each other or to other compounds;

b. X and Y are the same or different and each represents a chemical bond or one or more atoms of carbon, nitrogen, sulfur, oxygen or combinations thereof;

c. R and R' are the same or different and each is an alkyl, cycloalkyl, aryl, aralkyl or alkaryl group or a substituted or heterocyclic derivative thereof, wherein R and R' may be different in adjacent n units and have a selected stereochemical arrangement about the carbon atom to which they are attached;

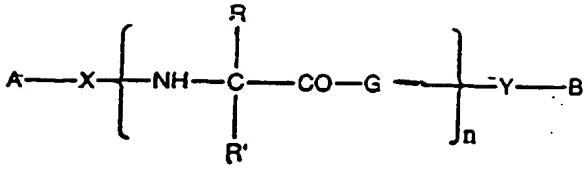
d. G is a connecting group or a chemical bond which may be different in adjacent n units; and

e. $n \geq 1$.

Preferably, (1) if n is 1, and X and Y are chemical bonds, A and B are different and one is other than a chemical bond, H or R; (2) if n is 1 and Y is a chemical bond, G includes a NH, OH or SH terminal group for connection to the carbonyl group and G-B is other than an amino acid residue or a peptide; (3) if n is 1 and X, Y, and G each is a chemical bond, A and B each is other than a chemical bond, an amino acid residue or a peptide; and (4) if n is 1, either X or A has to include a CO group for direct connection to the NH group.

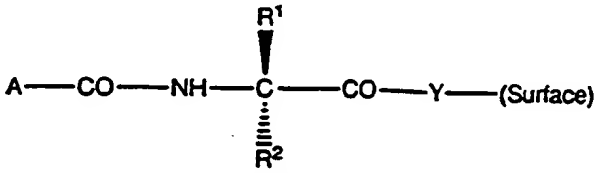
These compositions may be used to mimic various compounds such as peptides, nucleotides, carbohydrates, pharmaceutical compounds, reporter compounds, polymerizable compounds or substrates.

Another composition is defined by the formula:

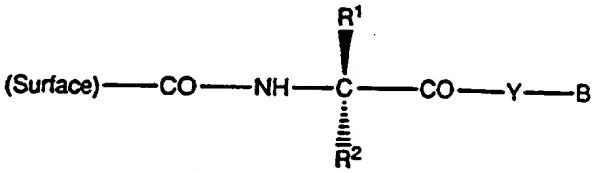


where A, B, X, Y and G are as defined above.

In one embodiment of the invention, at least one of A and B represents an organic or inorganic macromolecular surface functionalized with hydroxyl, sulfhydryl or amine groups. Examples of preferred macromolecular surfaces include ceramics such as silica and alumina, porous or nonporous beads, polymers such as a latex in the form of beads, membranes, gels, macroscopic surfaces, or coated versions or composites or hybrids thereof. A general structure of a chiral form of these materials is shown below:



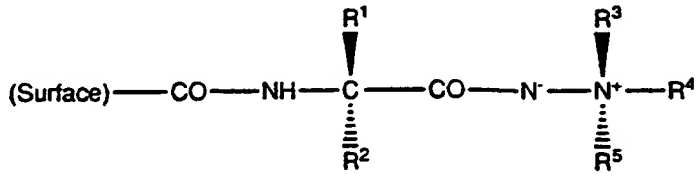
In another embodiment of the invention, the roles of A and B in the structure above are reversed, so that B is a substituent selected from the list given above and A represents a functionalized surface as shown for one of the enantiomeric forms:



In the description that follows, R^n where n = an integer will be used to designate a group from the definition of R and R^1.

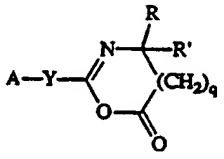
In a preferred embodiment, group A or B in the above structure is an aminimide moiety. This moiety may be introduced, for example by reacting the oxazolone with an asymmetrically substituted hydrazine and alkylating

the resulting hydrazide, (e.g., by reaction with an alkyl halide, or epoxide). An example of such a surface is shown below.



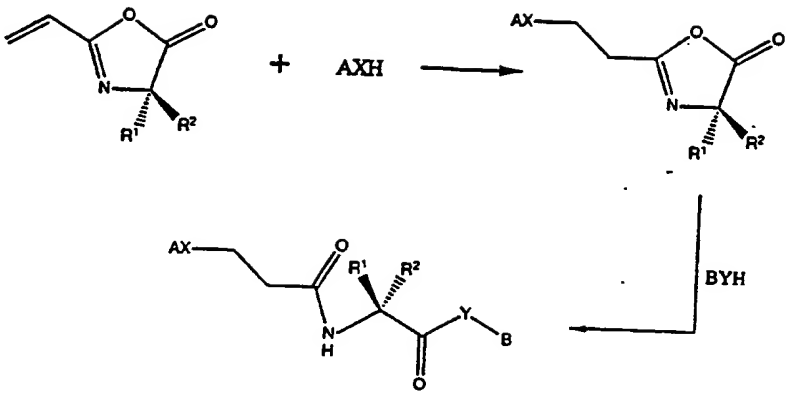
Preferred aminimides are described in a PCT application entitled MODULAR DESIGN AND SYNTHESIS OF AMINIMIDE-BASED MOLECULES USEFUL AS MOLECULAR RECOGNITION AGENTS AND NEW POLYMERIC MATERIALS (attorney docket no.: 5925-005-228) and filed of even date herewith, the content of which is expressly incorporated herein by reference thereto.

Another embodiment of the invention relates to an oxazolone ring having the structure



where A, R, R' and Y are as described above and q is zero or 1. Preferably, Y is a chemical bond [**see claim 36**]. This ring is useful for preparing the desired oxazolone derivatives.

A further embodiment of the invention exploits the capability of oxazolones with suitable substituents at the 2-position to act as alkylating agents. Appropriate substituents include vinyl groups, which make the oxazolone a Michael acceptor, haloalkyl and alkyl sulfonate-ester and epoxide groups. For example, Michael addition to the double bond of a chiral 2-vinyloxazolone followed by a ring opening reaction results in a chiral conjugate structure. This general reaction scheme, illustrated for the case of a 2-vinyl azlactone derivative, is as follows:



wherein X represents a sulfur or nitrogen atom; Y represents a sulfur, oxygen, or nitrogen atom; and substituents A and B, as described above, may adopt a variety of structures, differing markedly in their physical or functional properties or being the same, may be chiral or achiral, and may be preferably selected from amino acids, oligopeptides, polypeptides and proteins, nucleotides, oligonucleotides, ligand mimetics, carbohydrates, aminimides, or structures found in therapeutic agents, metabolites, dyes, photographically active chemicals, or organic molecules having desired steric, charge, hydrogen-bonding or hydrophobicity characteristics, or containing polymerizable vinyl groups.

The Michael reaction described above is usually carried out using stoichiometric amounts of nucleophile AXH and the oxazolone in a suitable solvent, such as toluene, ethyl acetate, dimethyl formamide, an alcohol, and the like. The product of the Michael addition is preferably isolated by evaporating the reaction solvent *in vacuo* and purifying the material isolated using a technique such as recrystallization or chromatography. Gravity- or pressure-chromatography, on one of a variety of supports, e.g., silica, alumina, under normal- or reversed-phase conditions, in the presence of a suitable solvent system, may be used for purification.

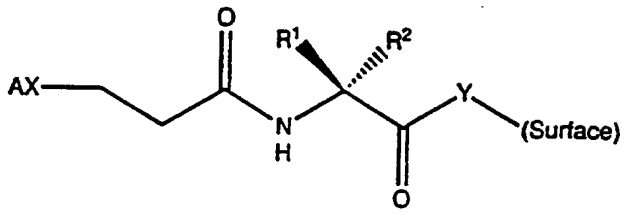
The selectivities of the Michael and oxazolone

ring-opening processes impose certain limitations on the choice of AXH and BYH nucleophiles shown above. Specifically, nucleophiles of the form ROH tend to add primarily via the ring-opening reaction, and often require acidic catalysts (e.g., BF_3); thus, X should not be oxygen. Likewise, primary amines tend to add only via ring-opening, and X should therefore not be NH. Secondary amines readily add to the double bond under appropriate reaction conditions, but many can also cause ring-opening; accordingly, X or Y can be N provided A or B are not hydrogen. Nucleophiles of the form RSH will exclusively add via ring-opening if the sulfhydryl group is ionized (*i.e.*, if the basicity of the reaction mixture corresponds to pH $\cong$ 9); on the other hand, such nucleophiles will exclusively add via Michael reaction under non-ionizing (i.e., neutral or acidic) conditions. During the Michael addition, it is important to limit the presence of hydroxylic species in the reaction mixture (e.g., moisture) to avoid ring-opening side-reactions.

Summarizing, AXH can be a secondary amine or thiol, and BYH can be a primary or secondary amine or thiol, or an alcohol.

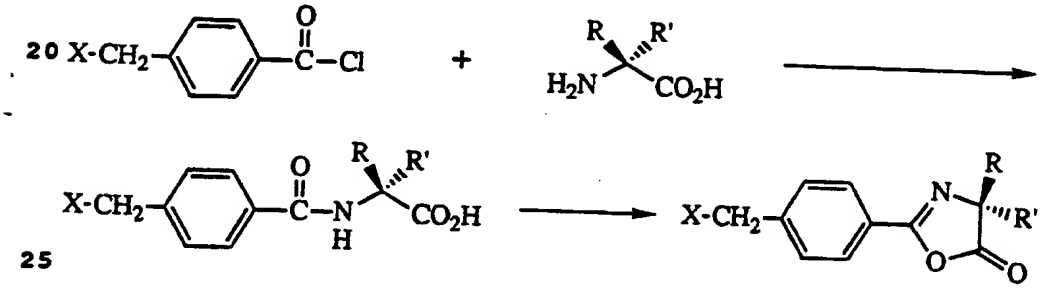
In one variant of the Michael-ring-opening sequence given above, A is a substituent selected from the foregoing list and BXH comprises an organic or inorganic macromolecular surface, e.g., a ceramic, a porous or non-porous bead, a polymer such as a latex in the form of a bead, a membrane, a gel or a composite, or hybrid of these; the macromolecular surface is functionalized with hydroxyl, sulfhydryl or amine groups which serve as the nucleophiles in the ring-opening reaction. The reaction sequence is carried out under conditions similar to those given for the nonpolymeric cases; purification of the final product involves techniques used in the art to purify supports and ther surfaces after derivatization, such as washing, dialysis,

etc. The result of this reaction sequence is a structure such as the one shown below:

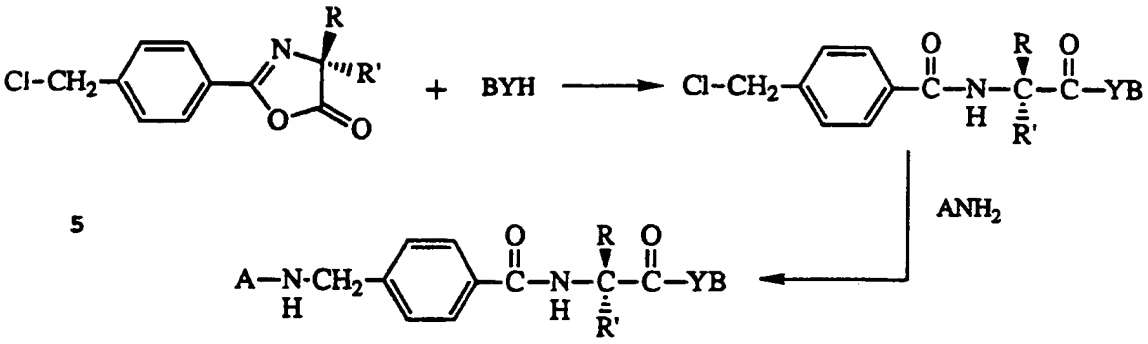


In another variant, the roles of AXH and BYH are reversed, so that BYH is the substituent selected from the list above and AXH represents a functionalized surface.

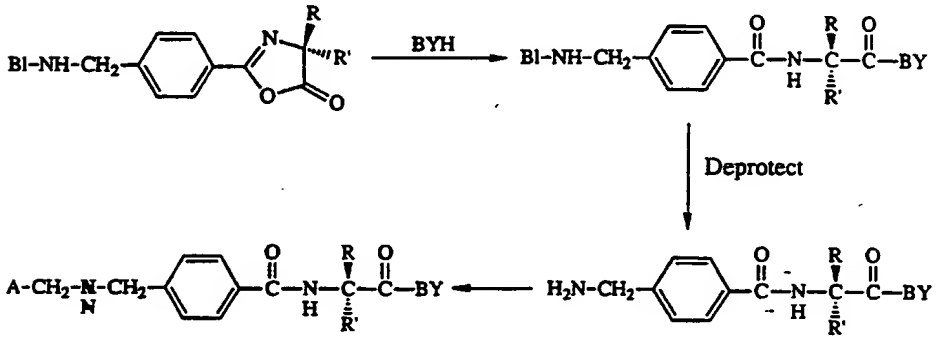
Alternatively, reactive groups may be introduced at the 2-position of the oxazolone ring *via* suitable acylations, as shown for the specific example of a benzoyl chloride derivative:



In the case where X is part of a group whose reactivity is orthogonal to that of the oxazolone ring, such as in the case of a benzyl chloride group, ring-opening addition with BYH may be carried out and followed by reaction with an appropriate AXH group, e.g. an amine ANH_2, to give the product shown:



If in the above sequences the benzylic electrophile competes with the oxazolone ring for the nucleophile BYH, a suitable protecting group, shown as Bl below, may be used to block an existing benzylic amino group in the oxazolone; subsequent to the ring-opening addition of BYH the protected group is removed using standard techniques (e.g., if the protecting group is Boc, it is removed by using dilute TFA in CH_2Cl_2), and the resulting product is reacted with an appropriate electrophile, e.g., A-CH_2-Br, thus introducing substituent A into the molecule.



4.2.2 Catenation of Chiral Oxazolones Producing Chiral Polymers

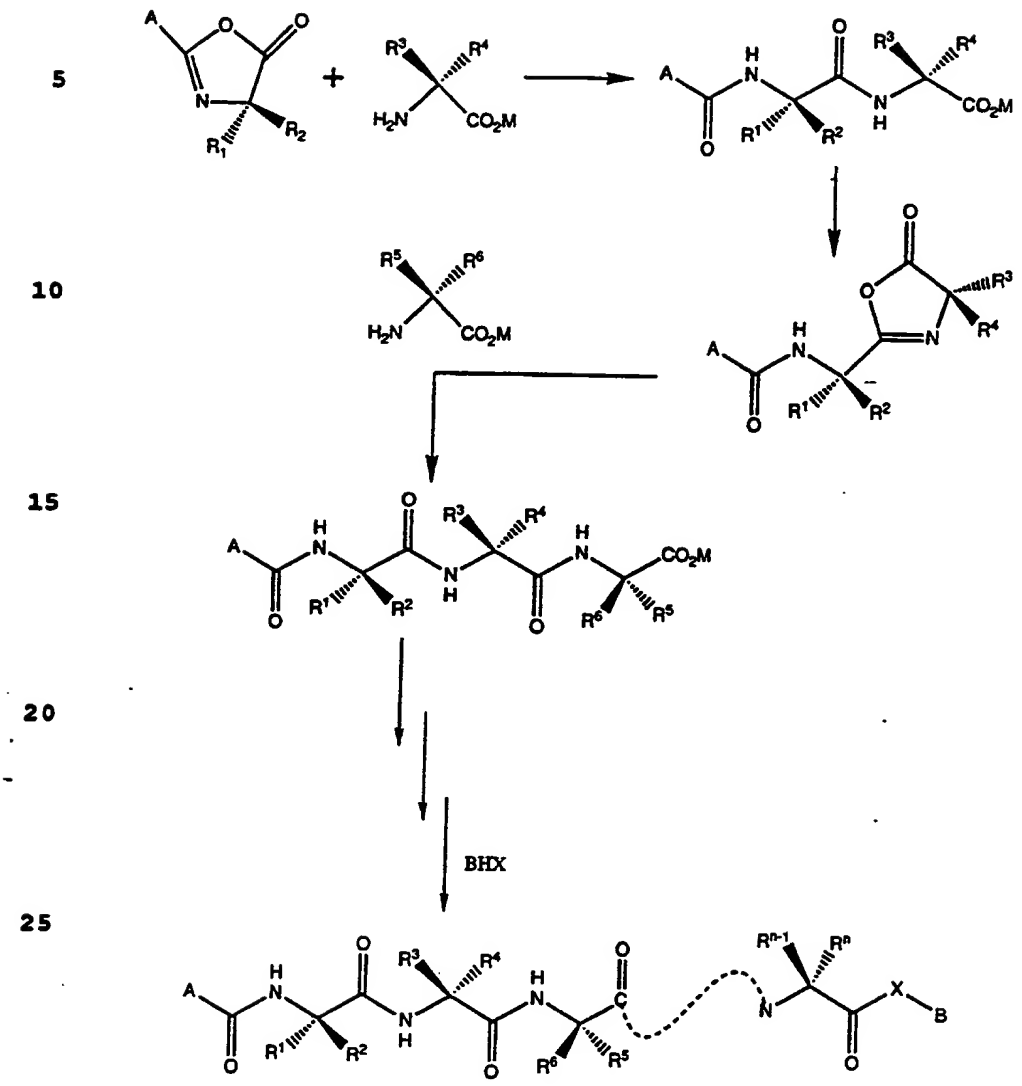
By selecting appropriate oxazolone building blocks and catenating (linking) them in one of a variety of ways, it is possible to produce polymeric functionalized scaffolds, of varying length and complexity, each of which mimicks a biologically important ligand and moreover possesses features which are desired of potent drugs, such as stability in physiological media, superior pharmacokinetics, etc. The oxazolones selected for catenation contain functional groups which, when part of the oxazolone-derived scaffold, will make specific contributions to the ligand-acceptor binding interaction, as determined by previous structural studies on the binding interaction.

Alternatively, by the judicious insertion of one or more oxazolone-derived units into a sequence of a peptide or protein, that is susceptible to hydrolysis or to enzymatic degradation, a hybrid molecule may be produced which has improved stability properties. These structures may be represented through the general conjugate structure given above; A and B represent the polypeptide sequences flanking the inserted oxazolone-derived unit or units.

The polymeric, oxazolone-derived ligand sequences may be constructed in one of three ways as outlined below.

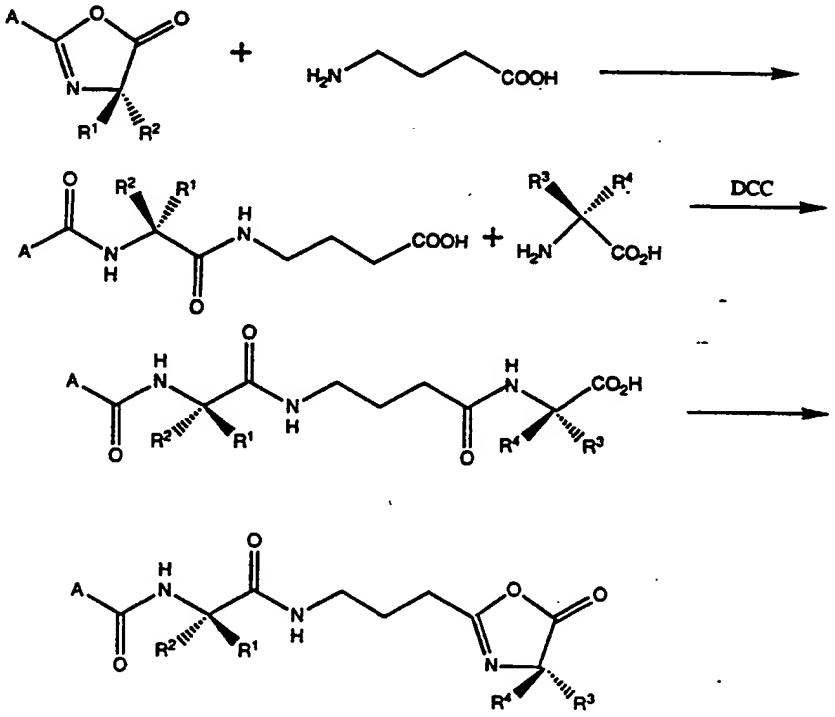
4.2.2.1 Polymerization Via Sequences of Nucleophilic Oxazolone-Ring-Opening Followed by Oxazolone-Forming Cyclization

According to this approach, the oxazolone ring is opened via nucleophilic attack by the amino group of a chiral α,α'-disubstituted amino acid; the resulting amide may be recyclized to the oxazolone, with retention of chirality, and subjected to a further nucleophilic ring-opening reaction, producing a growing chiral polymer as shown below:


BHX

wherein M is an alkali metal; each member of the substituent pairs R^1 and R^2, R^3 and R^4, and R^5 and R^6 differs from the other and taken alone each signifies alkyl, cycloalkyl, or substituted versions thereof, aryl, aralkyl or alkaryl, or substituted and heterocyclic versions thereof; these substituent pairs can also be joined into a carbocyclic or heterocyclic ring; preferred versions of these substituents are those mimicking side-chain structures found in naturally-occurring amino acids; X represents an oxygen, sulfur, or nitrogen atom; and A and B are the substituents described above.

At any point in the polymer synthesis shown above, a structural species, possessing (1) a terminal -OH, -SH or $-NH_2$ group capable of ring-opening addition to the oxazolone and (2) another terminal group capable of reacting with the amino group of a chiral α,α'-disubstituted amino acid, may be inserted in the polymer backbone as shown below:

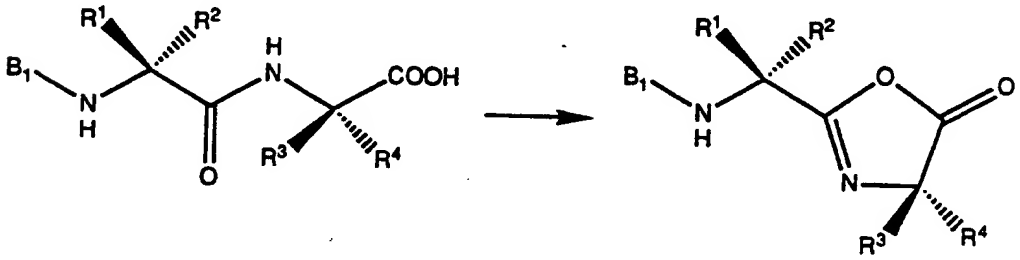


This process may be r peated, if desired, at each step in the synthesis where an oxazolone ring is produced. The bifunctional species used may be the same or different in the steps of the synthesis.

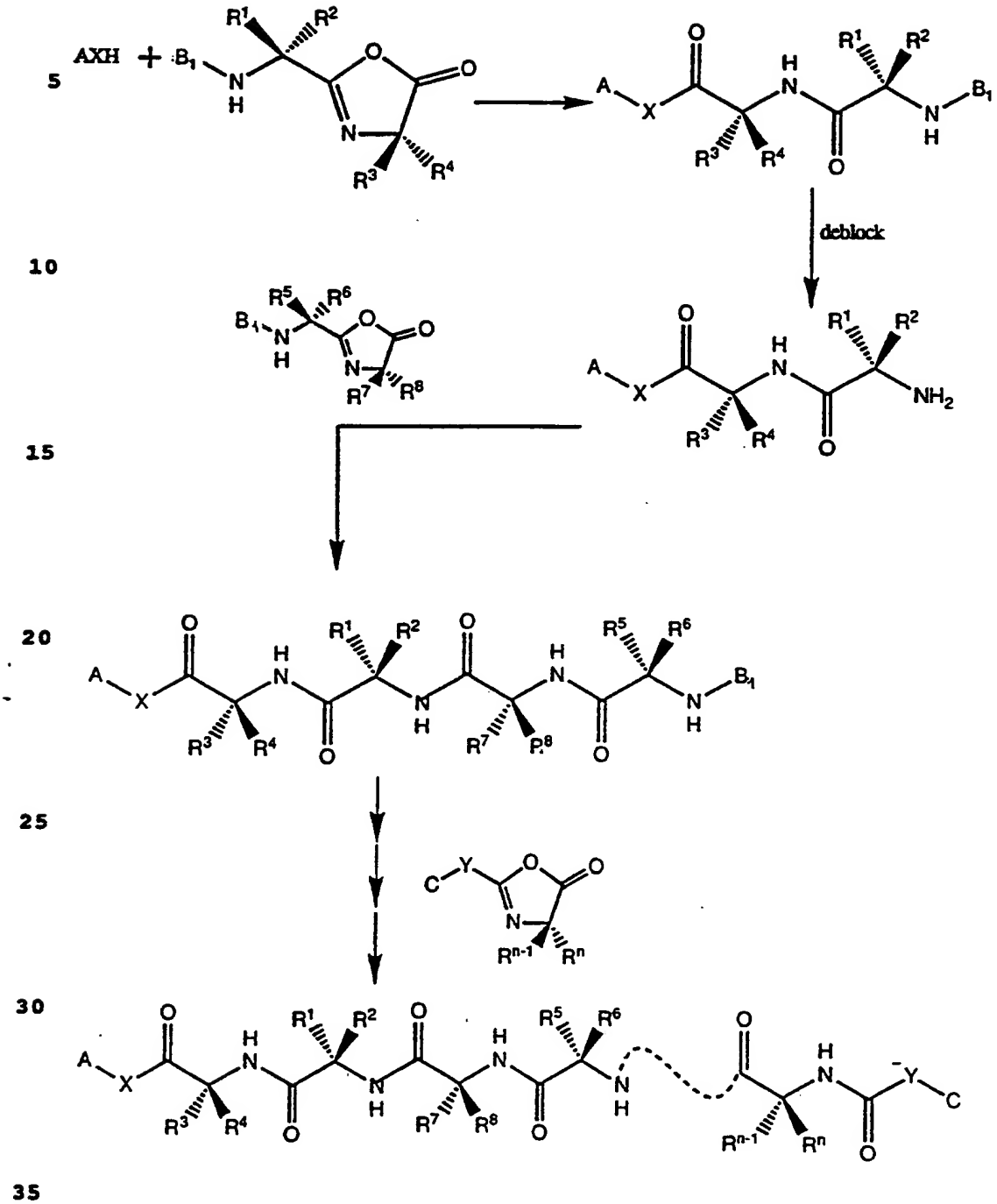
The experimental procedures described above for oxazolone formation and use of oxazolones as acylating agents are expected to be useful in the oxazolone-directed catenations. Solubility and coupling problems that may arise in specific cases can be dealt with effectively by one with ordinary skill in the art of polypeptide and peptide mimetic synthesis. For example, special solvents such as dipolar aprotic solvents (e.g., dimethyl formamide, DMF, dimethyl sulfoxide, DMSO, N-methyl pyrolidone, etc.) and chaotropic (molecular aggregate-breaking) agents (e.g., urea) will be very useful as catenations produce progressively larger molecules.

4.2.2.2 Polymerizations Using Bifunctional Oxazolones Containing a Nucleophilic Group

Alternatively, a chiral oxazolone derivative containing a blocked terminal amino group may be prepared from a blocked, disubstituted dipeptide, that was prepared by standard techniques known to those skilled in the art, as shown:

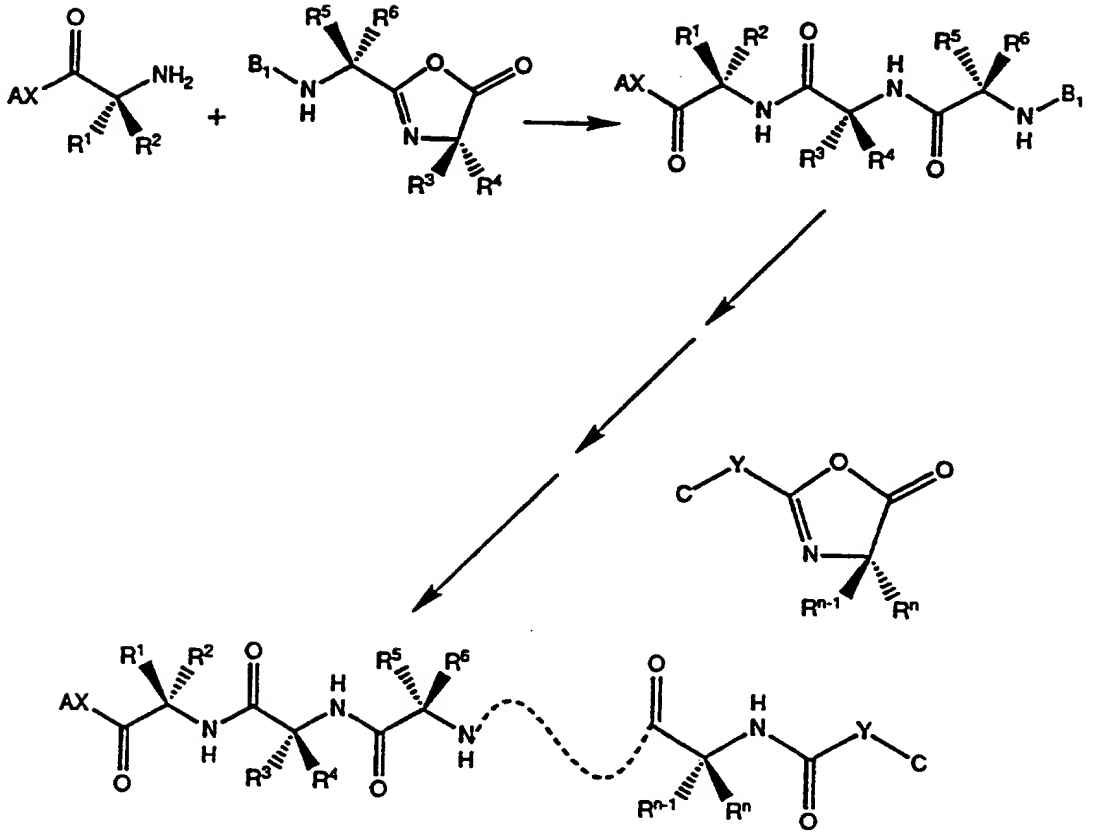


wherein B_1 is an appropriate protecting group, such as Boc (t-butoxycarbonyl) or Fmoc (fluorenylmethoxycarbonyl). One may then use this oxazolone to acylate an amine, hydroxyl, or sulfhydryl-group in a linker structure or functionalized solid support, represented generically by AXH, using the reaction conditions described above. This acylation is followed by deblocking, using standard amine deprotection techniques compatible with the overall structure of the amide (i.e., the amine protecting group is orthogonal with respect to any other protecting or functional groups that may be present in the molecule), and the resulting amino group is used for reaction with a new bifunctional oxazolone generating a growing chiral polymeric structure, as shown below:


deblock

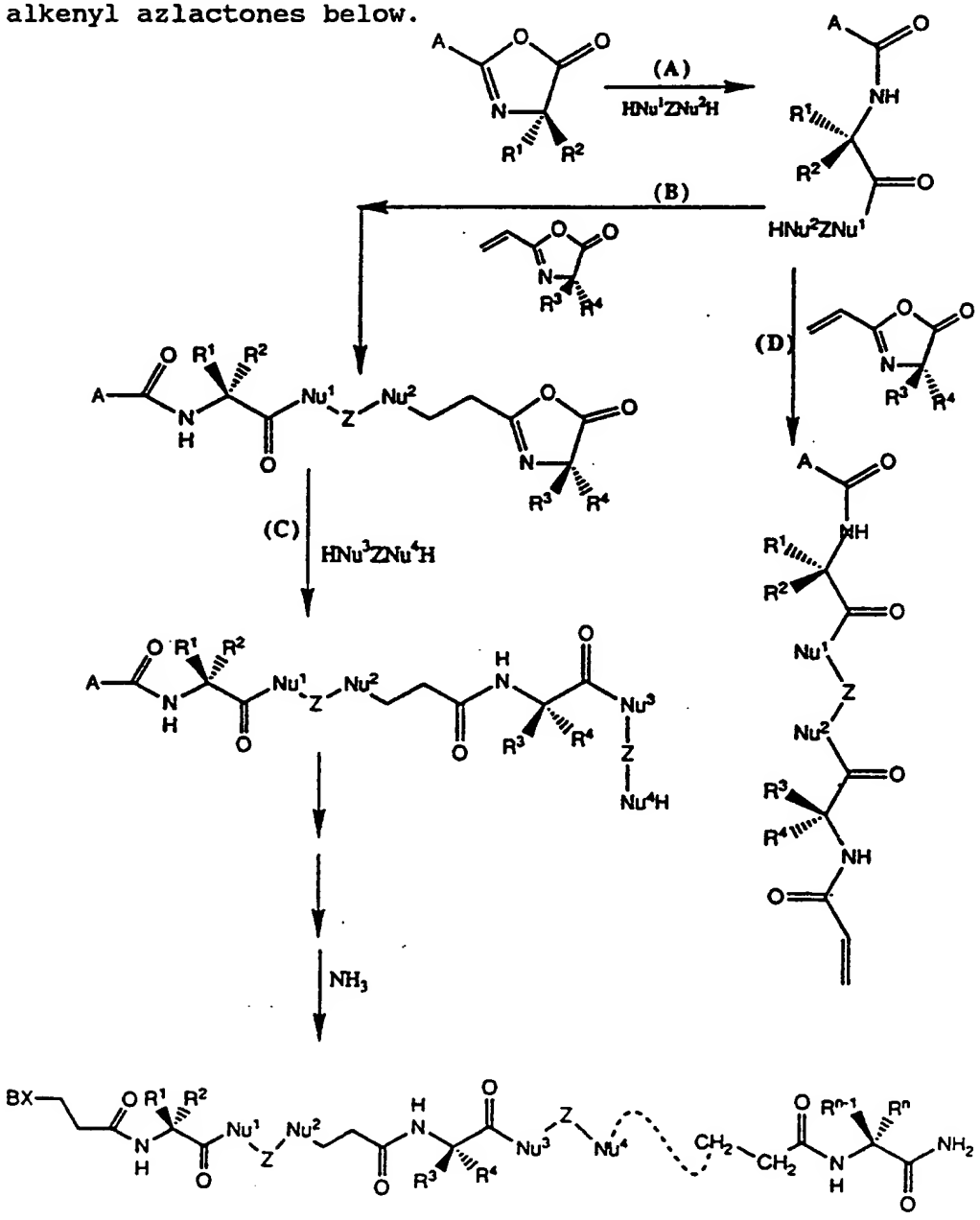
In the reaction shown above, Y is a linker (preferably a functionalized alkyl group); X is a nitrogen of suitable structure; an oxygen or a sulfur atom; each member of the substituent pairs R^1 and R^2, R^3 and R^4, R^{n-1} and R^n differs from the other and taken alone each signifies alkyl, cycloalkyl, or functionalized versions thereof; aryl, aralkyl or alkaryl or functionalized including heterocyclic versions thereof (preferably, these R substituents mimick the side-chain of naturally occurring amino acids); substituent R can also be part of a carbocyclic or heterocyclic ring; A is a substituent as described above; and C is a substituent selected from the set of structures for A; and B_1 is a blocking or protecting group.

It can be seen that the above polymerization involves introduction of two amino acid residues per polymer-elongation cycle and therefore produces ligands with an even number of residues. To obtain ligands containing an odd number of residues, a preliminary step may be carried out with a suitable amino acid derivative as shown below, prepared via standard synthesis.



4.2.2.3 Polymerization Using Bifunctional Oxazolones Containing an Additional Electrophilic Group

When the substituent at the 2-position of the oxazolone (azlactone) ring is capable of undergoing an addition reaction, that proceeds with retention of the chirality of the 4-position, the addition reaction may be combined with a ring-opening acylation to produce chiral polymeric sequences. This is shown for the case of alkenyl azlactones below.



In the above sequence of reactions, A denotes a structure of the form described above and HNu^1-Z-Nu^2H represents a structure containing two differentially reactive nucleophilic groups, such as methylamino-ethylamine, 1-amino propane-3-thiol, and so on; groups Nu^1, Nu^2, Nu^3 and Nu^4 need not be identical and Z is a linker structure as described above.

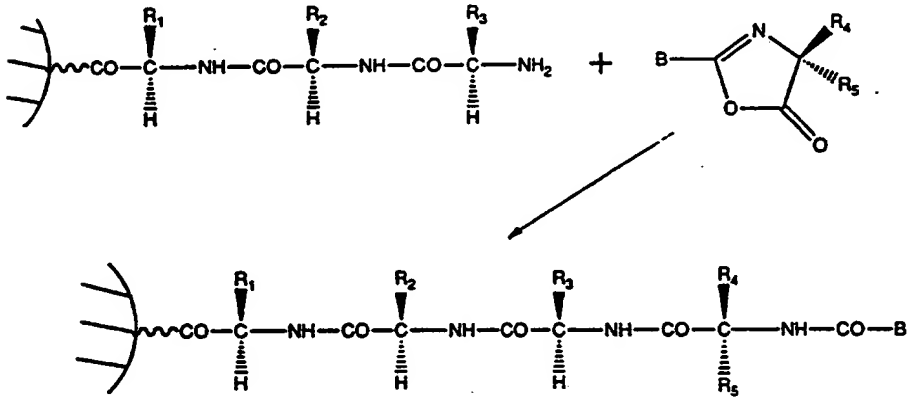
Structure HNu^1-Z-Nu^2H may contain two nucleophilic groups of differential reactivity, as stated above, or if Nu^1 and Nu^2 are of comparable reactivity one of the nucleophilic groups is protected to prevent it from competing with the other and deprotected selectively following acylation; protecting groups commonly used in the art of peptide synthesis (e.g., for the nucleophilic groups such as amino, hydroxyl, thio, etc.) are useful in the protection of one of the Nu substituents of the structure HNu^1-Z-Nu^2H. The product of the acylation reaction with HNu^1-Z-Nu^2H (after Nu-deprotection, if necessary) is further reacted with a new oxazolone unit in a Michael fashion, and this addition is followed by ring-opening acylation with an additional dinucleophile; repetition of this sequence of synthetic steps produces a growing polymeric molecule. Reaction conditions for carrying out these processes are similar to those described above for related polymers.

The above types of oligomers are highly useful biochemically because of their structural similarity to polypeptides. The substituents R can be chosen to tailor the steric, charge or hydrophobicity characteristics of the oligomer such that a versatile polypeptide mimetic results.

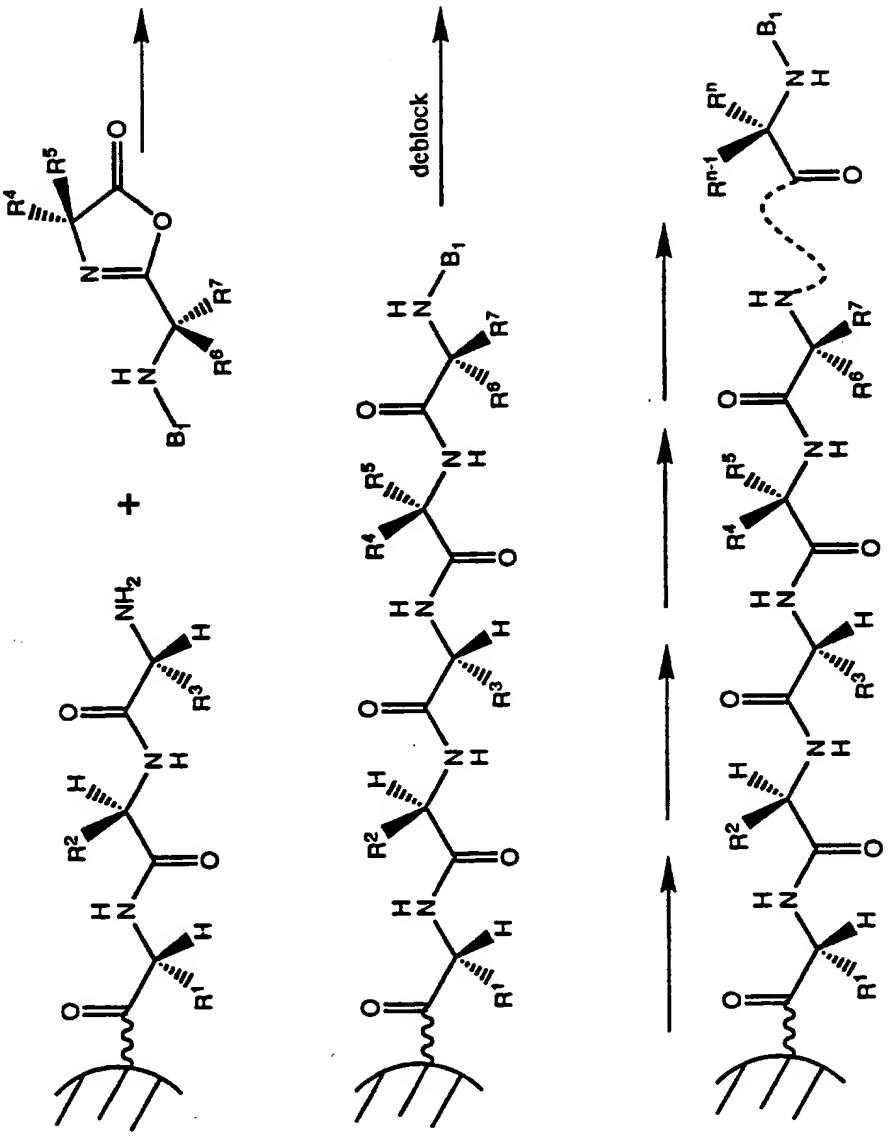
4.2.3 Functionalization of Peptides and Proteins Using Oxazolones

In a further embodiment of the invention, the nucleophilic ring-opening of asymmetrically disubstituted oxazolones may be utilized to introduce a chiral residue or sequence in selected positions in peptides or proteins to produce hybrid molecules with improved hydrolytic stability or other properties.

The reaction of a chiral azlactone with the amino terminus of a synthetic tripeptide attached to a Merrifield support is shown below.

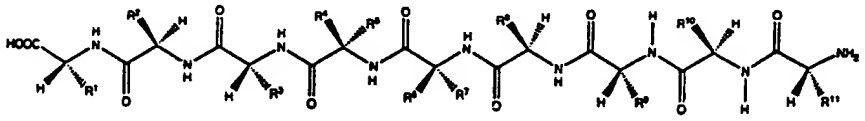


The oxazolone used in the above aminolysis may contain a blocked amino terminus which, after the aminolysis, is deblocked and used for further elongation via acylation. This synthetic variation is shown below (B_1 stands for a suitable blocking group as described above).


deblock

After the desired oxazolone units have been used to elongate a given polypeptide, the polypeptide synthesis may be continued, if desired, using standard peptide-synthesis techniques.

The structure below illustrates a short polymer containing nine subunits prepared as above and detached from the solid phase synthesis support.

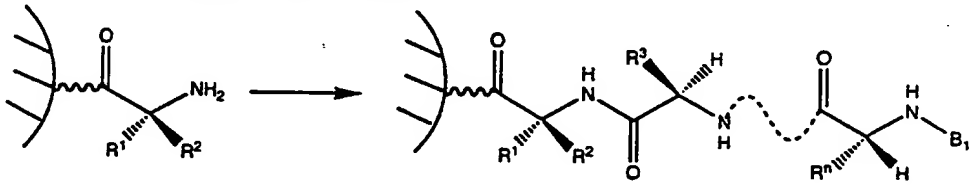


In the polyamide structure shown above, each of the R groups signifies alkyl, cycloalkyl, or substituted version thereof; aryl, aralkyl, alkaryl, or substituted including heterocyclic versions thereof; the R groups can also define a carbocyclic or heterocyclic ring; preferred structures for the R groups are those mimicking the structures of the side-chains of naturally-occurring amino acids.

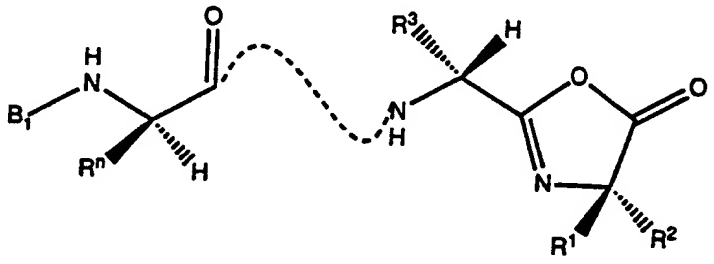
The syntheses outlined above may be carried out using procedures similar to those described previously for related molecules and macromolecules.

Alternatively, disubstituted chiral azlactones may be utilized to introduce a variety of novel, unnatural residues into peptides or proteins using the following multistep procedure:

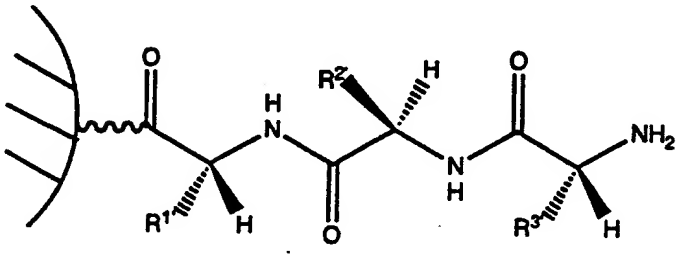
a. Synthesis of a peptide whose carboxyl terminal residue is chiral and disubstituted, preferably via solid phase synthesis:



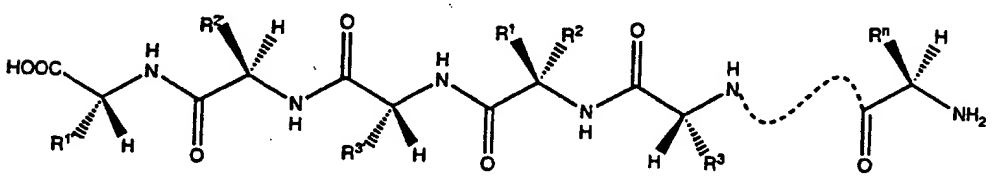
b. Detachment of the peptide prepared by solid phase synthesis from the support, with reblocking of the N-terminus if necessary, followed by cyclization producing the oxazolone as shown below:



c. Synthesis of a second desired peptide sequence on a solid support:



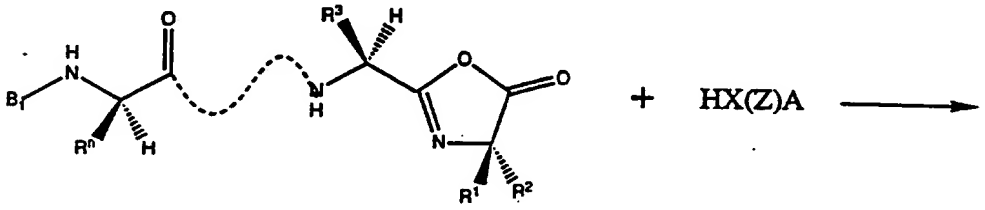
d. Coupling of the peptides produced in steps (b) and (c) above, under suitable reaction conditions, producing a novel peptide containing unnatural residues, shown below after detachment of the peptide from the support and removal of all protecting groups used during its synthesis.

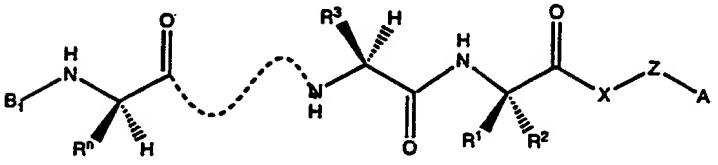


In the structure above, each of the R groups signifies alkyl, cycloalkyl, aryl, aralkyl or alkaryl, or substituted or suitably heterocyclic versions thereof; the R groups may also define a carbocyclic or heterocyclic ring; preferably the R groups are structural mimetics of the side-chains of naturally-occurring amino acids.

Again, the reactions shown in steps a-d above are carried out using the conditions described above for related cases. Couplings of peptide segments on a support or in solution are carried out using the traditional techniques from the field of peptide synthesis.

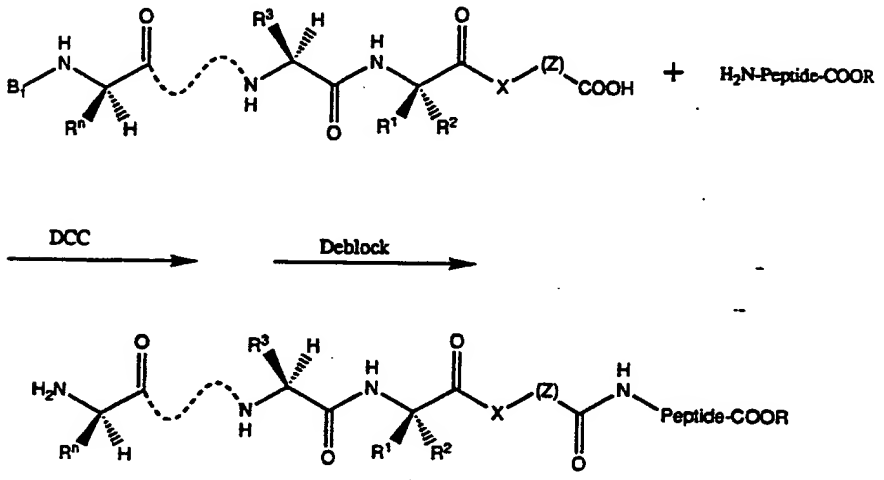
In a variation of the above synthesis, the oxazolone peptide produced in step (b) above may be reacted with a variety of bifunctional nucleophilic molecules to give acylation products as shown below:



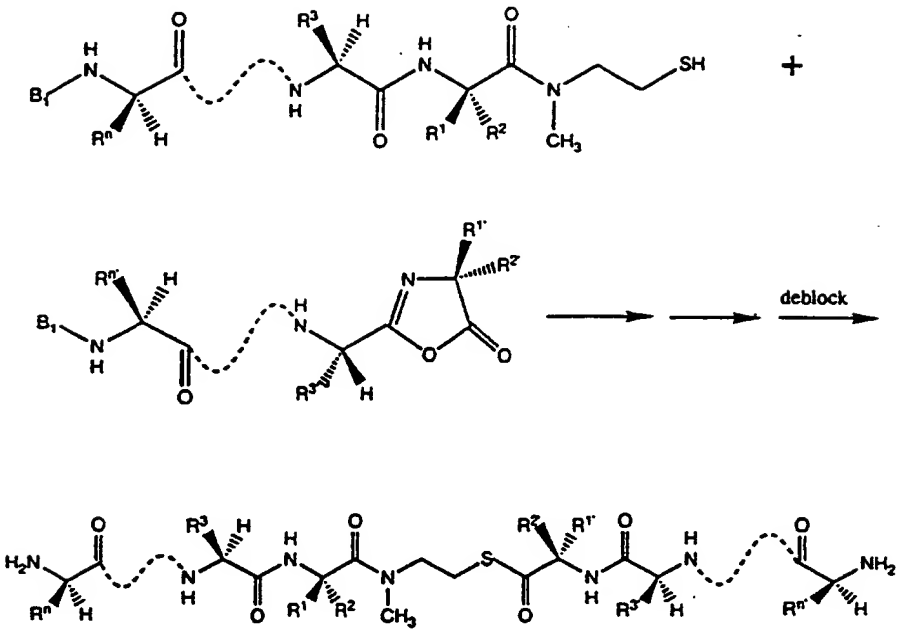


The above acylation product may be coupled with a peptide to produce novel chiral hybrids; two coupling routes may be used.

(1) If A is a group which can be condensed with an amino group, the condensation reaction is used for coupling. For example, if A is a carboxyl group, condensation with a peptide amine using DCC or similar reagent produces the desired product. Reaction conditions and suitable (orthogonal) protecting groups well-known in the art, such as those described above, are expected to be useful.



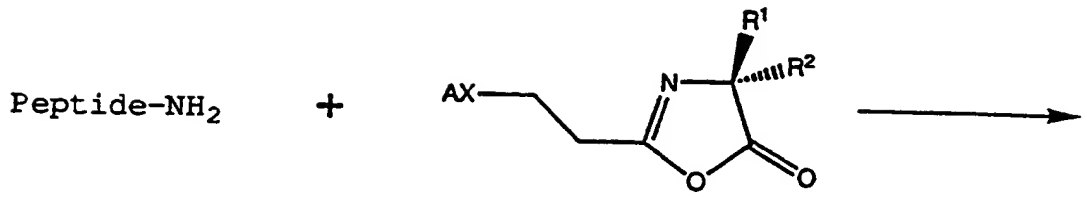
(2) If A is a suitable nucleophilic group (e.g., hydroxyl, amino, thio, etc.) it may be used to open a peptide oxazolone containing a protected amino terminus. In the case shown below, groups Y, A and Z of the general structure shown above have been defined as follows: Y = NCH_3, A = SH and Z = CH_2CH_2:

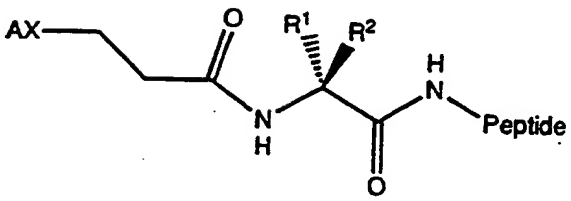


The above reactions are run under conditions, similar to those described above for related peptide syntheses. A great variety of molecules possessing nucleophilic hydroxyl, thio, amino and other groups, e.g., carbohydrates, may be conjugated with peptidic and related frameworks using reactions with suitable oxazolones as outlined above.

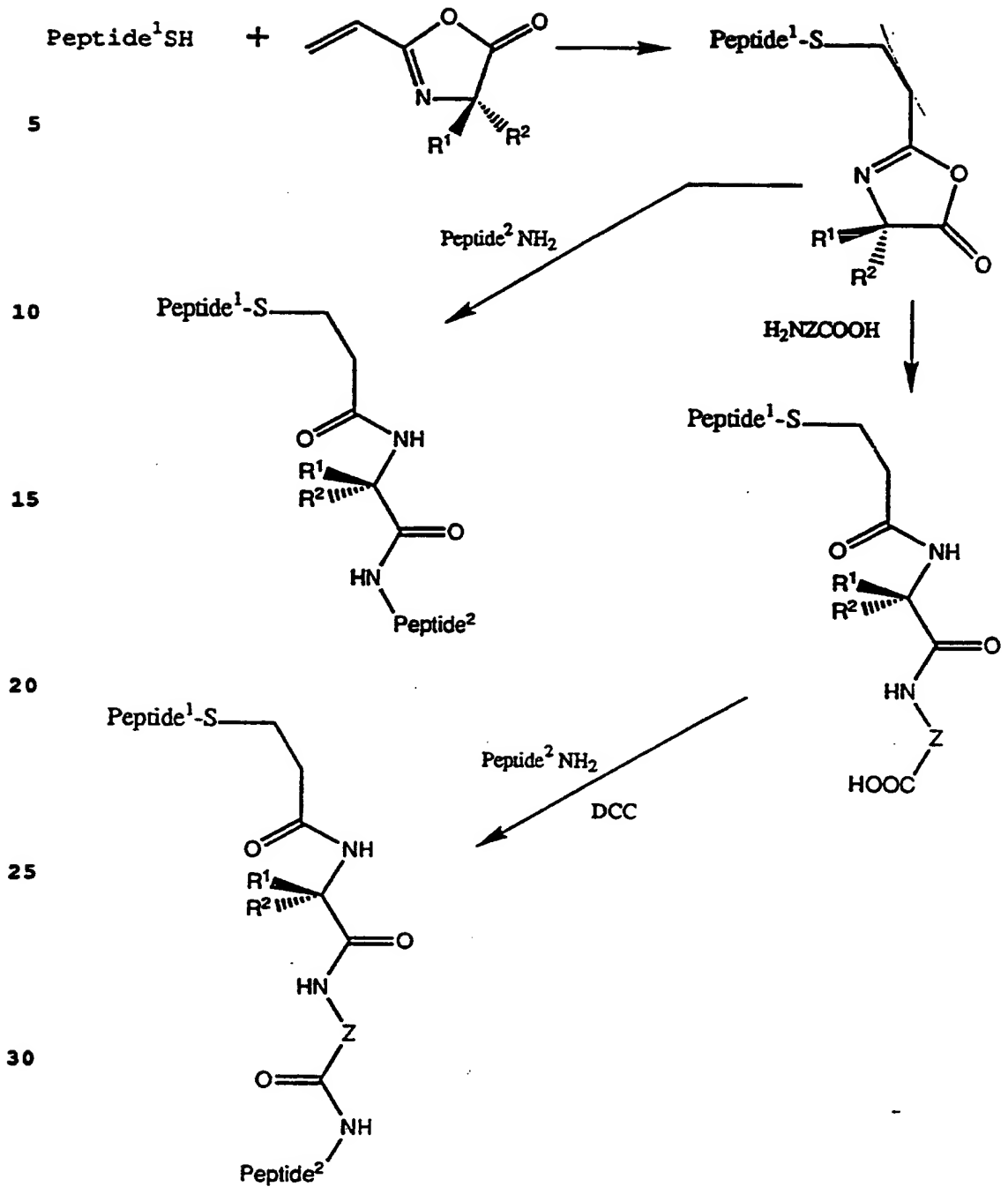
Alternatively, residues may be attached to or inserted into peptide chains using oxazolones with reactive groups attached at the 2-position of the ring. This may be accomplished in either of two ways, as illustrated below for the case of 2-alkenyl azlactones.

(1) Nucleophilic attack on an azlactone, that was previously derivatized via a Michael addition using a nucleophile of general structure AXH, with a peptide amine:





(2) Michael addition of a peptide nucleophile, e.g., a sulfhydryl group, to the double bond of a 2-vinyl oxazolone, followed by nucleophilic attack on the oxazolone ring by another peptide nucleophile, e.g., an amine followed by further modifications; this sequence produces polymeric molecules of a variety of structures as shown below:


Peptide1SH
+
Peptide1-S
Peptide2 NH2
H2NZCOOH
Peptide1-S
Peptide2 NH2
DCC
Peptide1-S
Peptide2

4.2.4 Fabrication of Ozaxolone-Derived Macromolecular Structures Capable of Specific Molecular Recognition

In an embodiment of the invention oxazolone molecular building blocks may be utilized to construct new macromolecular structures capable of recognizing specific molecules ("intelligent macromolecules"). These "intelligent macromolecules" may be represented by the following general formula:

P - C - L - R

where R is a structure capable of molecular recognition;
L is a linker;
P is a macromolecular structure serving as a supporting platform;
C is a polymeric structure serving as a coating which surrounds P.

Structure R may be a native ligand of a biological ligand-acceptor, or a mimetic thereof, such as those described above.

Linker L may be a chemical bond or one of the linker structures listed above, or a sequence of subunits such as amino acids, aminimide monomers, oxazolone-derived chains of atoms or the like.

Polymeric coating C may be attached to the supporting platform either via covalent bonds or "shrink wrapping," i.e., the bonding that results when a surface is subjected to coating polymerization well known to those skilled in the art. This coating element may be 1) a thin crosslinked polymeric film 10 - 50 Å in thickness, 2) a crosslinked polymeric layer having controlled microporosity and variable thickness, or 3) a controlled microporosity gel. When the support platform is a microporous particle or a membrane, as described

below, the controlled microporosity gel may be engineered to completely fill the porous structure of the support platform. The polymeric coatings may be constructed in a controlled way by carefully controlling a variety of reaction parameters, such as the nature and degree of coating crosslinking, polymerization initiator, solvent, concentration of reactants, and other reaction conditions, such as temperature, agitation, etc., in a manner that is well known to those skilled in the art.

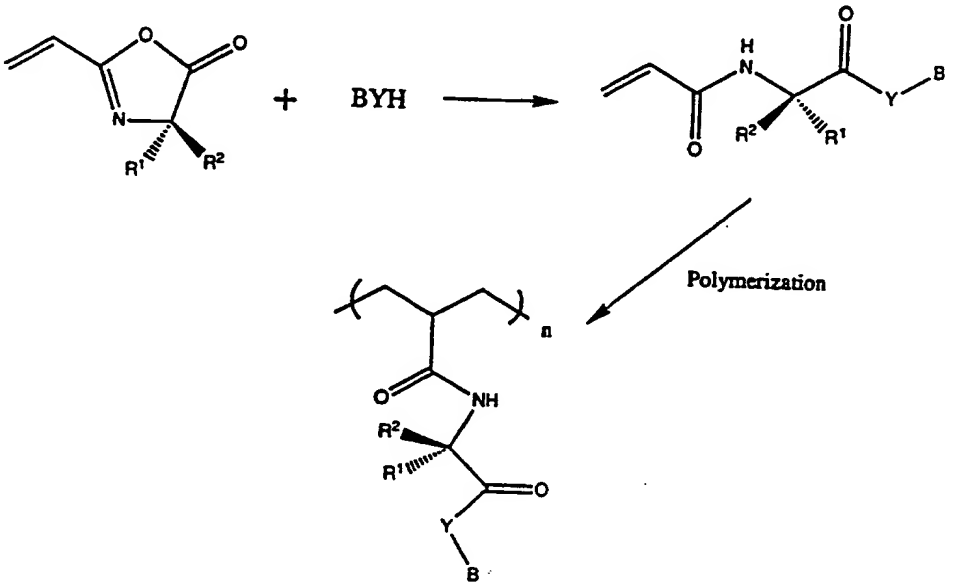
The support platform P may be a pellicular material having a diameter (dp) from 100 Å to 1000 μ, a latex particle (dp 0.1 - 0.2 μ), a microporous bead (dp 1 - 1000 μ), a porous membrane, a gel, a fiber, or a continuous macroscopic surface. These may be commercially available polymeric materials, such as silica, polystyrene, polyacrylates, polysulfones, agarose, cellulose, etc.

The multisubunit recognition agents above are expected to be very useful in the development of targeted therapeutics, drug delivery systems, adjuvants, diagnostics, chiral selectors, separation systems, and tailored catalysts.

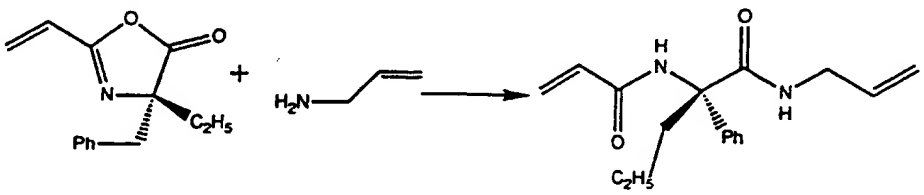
In the present specification the terms "surface", "substrate" or "structure" refer either to P, P linked to C or P linked to C and L as defined above.

4.2.4.1 Chiral Alkenyl Azlactone Monomers and Polymerization Products

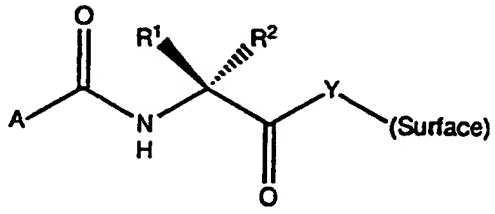
When used on an alkenyl azlactone, the azlactone ring-opening addition reaction discussed above may be used to directly produce a wide variety of chiral vinyl monomers. These may be polymerized or copolymerized to produce chiral oligomers or polymers, and may be further crosslinked to produce chiral beads, membranes, gels, coatings or composites of these materials.

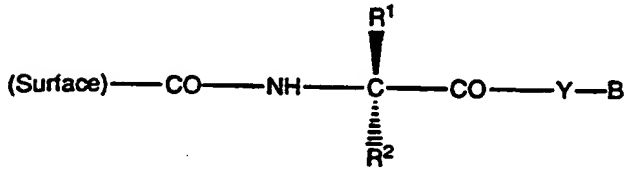


Other useful monomers, which may be used to produce chiral crosslinkable polymers, may be produced by nucleophilic opening of a chiral 2-vinyl oxazolone with a suitable amino alkene or other unsaturated nucleophile.



Vinyl polymerization and polymer-crosslinking techniques are well-known in the art (see, e.g., U.S. Patent No. 4,981,933) and are applicable to the above preferred processes.





4.2.5 Combinatorial Libraries of Peptidomimetics Derived From Oxazolone Modules

The synthetic transformations of oxazolones outlined above may be readily carried out on solid supports in a manner analogous to performing solid phase peptide synthesis, as described by Merrifield and others (see for example, Barany, G., Merrifield, R.B., Solid Phase Peptide Synthesis, in The Peptides Vol. 2, Gross E., Meienhofer, J. eds., p. 1-284, Acad. Press, New York 1980; Stewart, J.M., Yang, J.D., Solid Phase Peptide Synthesis, 2nd ed., Pierce Chemical Co., Rockford, Illinois 1984; Atherton, E., Sheppard, R.C., Solid Phase Peptide Synthesis, D. Rickwood & B.D. Hames eds., IRL Press ed. Oxford U. Press, 1989). Since the assembly of the oxazolone-derived structures is modular, *i*.e., the result of serial combination of molecular subunits, huge combinatorial libraries of oxazolone-derived oligomeric structures may be readily prepared using suitable solid-phas chemical synthesis techniques, such as those of

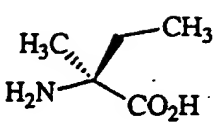
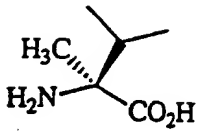
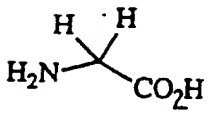
described by Lam (K.S. Lam, et al. Nature 354, 82 (1991)) and Zuckermann (R.N. Zuckermann, et al. Proc. Natl. Acad. Ser. USA, 89, 4505 (1992); J.M. Kerr, et al., J. Am Chem. Soc. 115, 2529 (1993)). Screening of these libraries of compounds for interesting biological activities, *e.g.*, binding with a receptor or interacting with enzymes, may be carried out using a variety of approaches well known in the art. With "solid phase" libraries (*i.e.*, libraries in which the ligand-candidates remain attached to the solid support particles used for their synthesis) the bead-staining technique of Lam may be used. The technique involves tagging the ligand-candidate acceptor, *e.g.*, an enzyme or cellular receptor of interest, with an enzyme (*e.g.*, alkaline phosphatase) whose activity can give rise to color prodution thus staining library support particles which contain active ligands-candidates and leaving support particles containing inactive ligand-candidates colorless. Stained support particles are physically removed from the library (*e.g.*, using tiny forceps tht are coupled to a micromanipulator with the aid of a microscope) and used to structurally identify the biologically active ligand in the library after removel of the ligand acceptor from the complex by *e.g.*, washing with 8M guanidine hydrochloride. With "solution-phase" libraries, the affinity selection techniques described by Zuckermann above may be employed.

An especially preferred type of combinatorial library is the encoded combinatorial library, which involves the synthesis of a unique chemical code (*e.g.*, an oligonucleotide or peptide), that is readily decipherable (*e.g.*, by sequencing using traditional analytical methods), in parallel with the synthesis of the ligand-candidates of the library. The structure of the code is fully descriptive of the structure of the ligand and used to structurally characterize biologically active ligands whose structures are difficult or

impossible to elucidate using traditional analytical methods. Coding schemes for construction of combinatorial libraries have been described recently (for example, see S. Brenner and R.A. Lerner, Proc. Natl. Acad. Sci. USA 89, 5381 (1992); J.M. Kerr, et al. J. Am. Chem. Soc. 115, 2529 (1993)). These and other related schemes are contemplated for use in constructing encoded combinatorial libraries of oligomers and other complex structures derived from oxazolones.

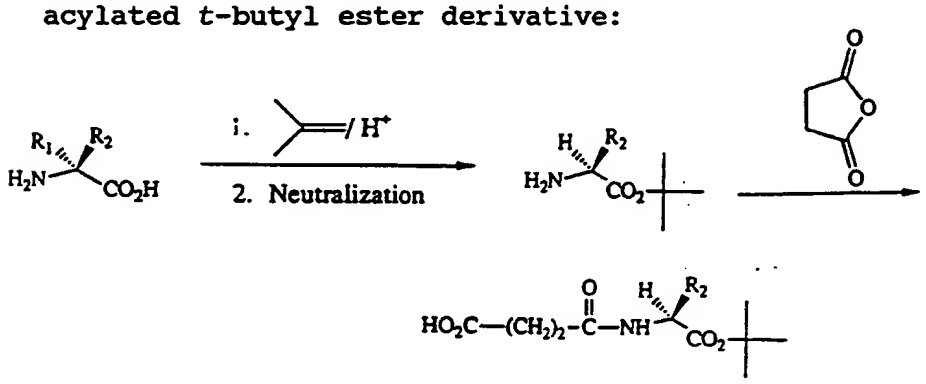
The power of combinatorial chemistry in generating screenable libraries of chemical compounds *e.g.*, in connection with drug discovery, has been described in several publications, including those mentioned above. For example, using the "split solid phase synthesis" approach outlined by Lam et al, the random incorporation of 20 oxazolones into pentameric structures, wherein each of the five subunits in the pentamer is derived from one of the oxazolones, produces a library of 20^5 = 3,200,000 peptidomimetic ligand-candidates, each ligand-candidate is attached to one or more solid-phase synthesis support particles and each such particle contains a single ligand-canditate type. This library can be constructed and screened for biological activity in just a few days. Such is the power of combinatorial chemistry using oxazolone modules to construct new molecular candidates.

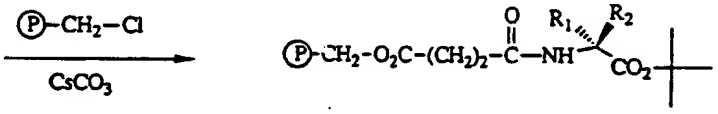
The following is one of the many methods that are being contemplated for use in constructing random combinatorial libraries of oxazolone-derived compounds; the random incorporation of three oxazolones derived from the amino acids glycine methyl-ethyl-glycine ,and isopropyl methyl glycine to produce 27 trimeric structures, linked to the support via a succinoyl linker is given as an example.

(1) A suitable solid phase synthesis support, *e.g.*, the chloromethyl resin of Merrifield, is split into three equal portions.

(2) Each portion is coupled to one and one of the glycines shown above after conversion to the acylated *t*-butyl ester derivative:



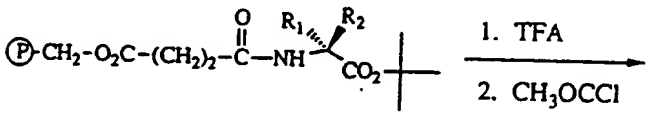


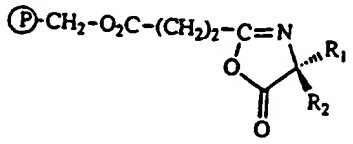
Ⓟ = polystyrene
R_1, R_2 = H, CH_3, CH_2CH_3, $(CH_3)_2CH$

The conditions for carrying out the above transformations are well known and used routinely in the art of peptide synthesis as described in the references given above.

(3) Each amino acyl resin portion is treated with an acid solution such as neat trifluoroacetic acid (TFA), or preferably, a 1:1 mixture of TFA and

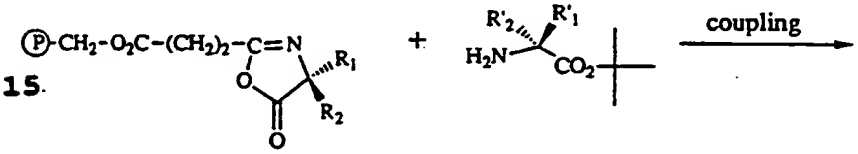
CH_2Cl_2, to remove the *t*-Bu blocking group. The resulting acyl amino acid resin is treated with ethyl chloroformate as described above producing the oxazolone resin.

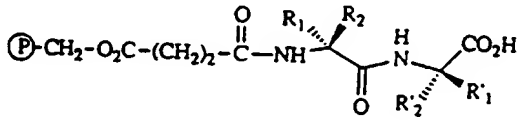


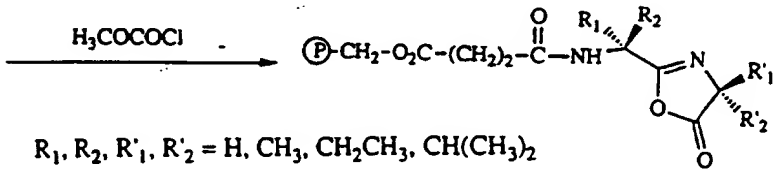


(4) The three oxazolone resin portions are thoroughly mixed and the resulting mixture is split into three equal portions.

(5) Each of the resin portions is coupled to a different glycine protected as *t*-butyl ester using the conditions described above; the amide product is deprotected as described above, for each of the resin portions and cyclized to the oxazolone using the reaction with ethyl chloroformate.



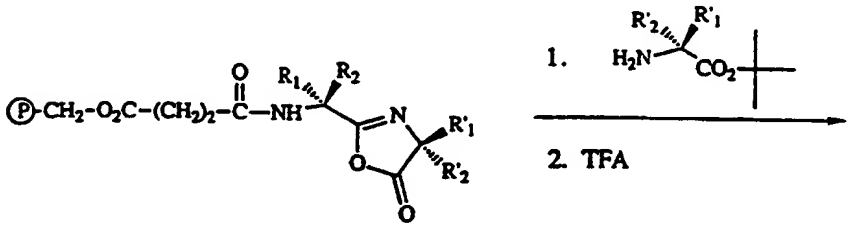


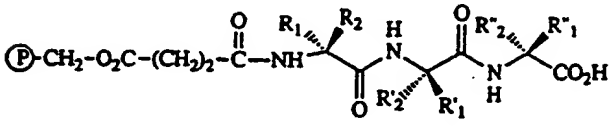


R_1, R_2, R'_1, R'_2 = H, CH_3, CH_2CH_3, $CH(CH_3)_2$

(6) The resulting resin portions are mixed thoroughly and then split again into three equal portions.

(7) Each of the resin portions is coupled to a different glycine, containing a carboxyl protected as the t-butyl ester, and the product is deprotected using TFA as described above; the resin portions are mixed producing a library containing 27 types of resin beads, each type containing a single oxazolone-derived tripeptide analog linked to the support via a succinoyl linker; this linker may be severed using acidolysis to produce a "solution-phase" library of peptides whose N-terminus is succinoylated.





Many modifications of this general scheme are envisioned, including the direct attachment of the ligand candidates via a C-N bond using a benzhydryl support, which would allow the straight forward detachment of the ligand candidates from the support via acidolysis for further study ("one-head, one-peptide-analog synthesis").

4.2.6.1 Design and Synthesis of Oxazolone-Derived Glycopeptide Mimetics

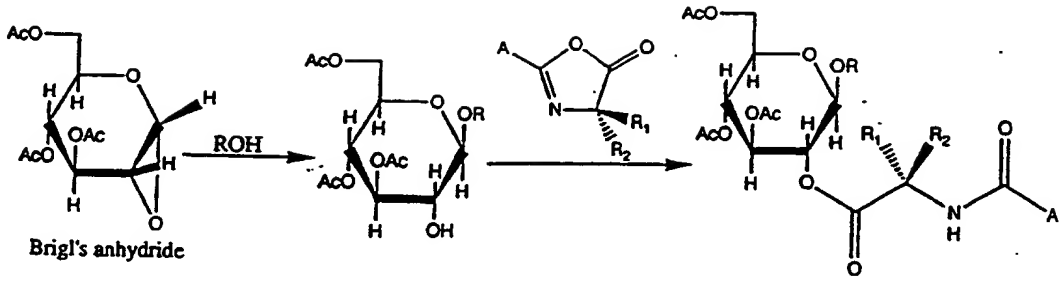
A great variety of saccharide and polysaccharide structural motifs incorporating oxazolone-derived structures are contemplated including but not limited to the following.

(1) Oxazolone-derived structures which mimic native peptide ligands capable of binding to saccharide and polysaccharide receptors using the design and synthesis techniques that are described above.

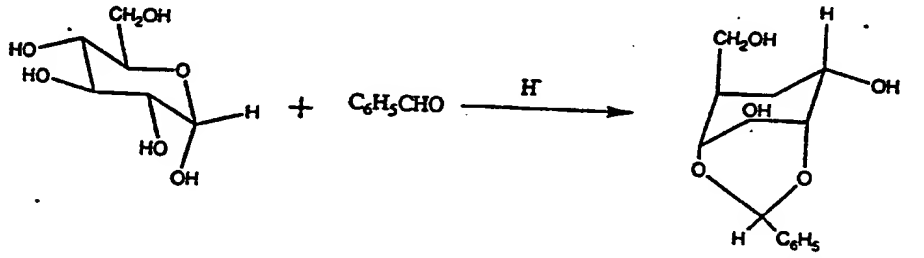
(2) Oxazolone-derived structures linking mono-, oligo- or polymeric saccharides with each other or with other structures capable of recognizing a ligand acceptor.

A wealth of chemical methods for synthesis of the above saccharides are available. The art of carbohydrate chemistry describes numerous sugars of variety of sizes with selectively blocked functional groups, which allows for selective reactions with oxazolone and related species producing the desired products (see Comprehensive Organic Chemistry, Sir Derek Barton, Chairman of Editorial Board, Vol. 5, E. Haslam, Ed., pp. 687-815; A. Streitwieser, C.H. Heathcock, E. Kosower, Introduction to Organic Chemistry, 4th Edition, MacMillan Publ. Co., New York, pp. 903-949.

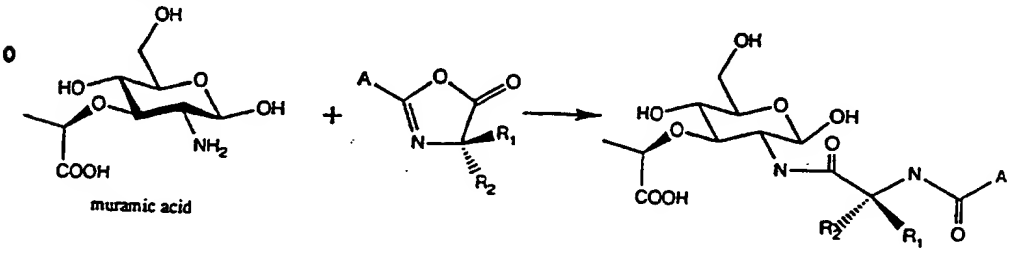
For example, Brigl's anhydride shown below can be reacted with unhindered alcohols to produce β-glucosides using well-known experimental conditions. The resulting sugar, blocked at all positions except position 2, can be used to open a suitable oxazolone using the reaction conditions described above, *e.g.*, in the absence or presence of a Lewis acid catalyst such as BF_3 in a suitable inert organic solvent (*e.g.*, EtOAC, dioxane, etc.).



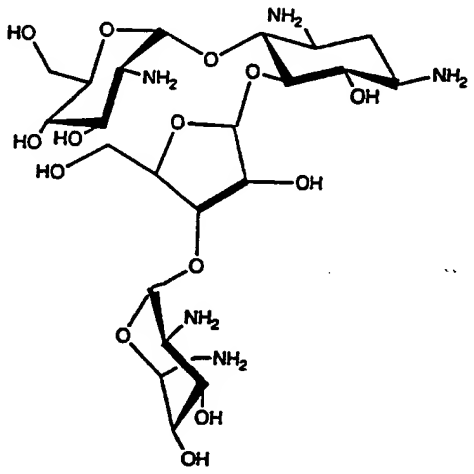
Similarly the sugar that results from reaction of D-glucose with benzaldehyde can be readily blocked at positions 1 and 6, by sequential reactions with an alcohol in the presence of acid, and tritylation using techniques well known in the art of carbohydrate chemistry. The resulting sugar, with position 3 unblocked can be used selectively as described above to derivatize a desired oxazolone structure.



A suitable oxazolone can also be ring-opened by a sugar containing reactive amino substituents, *i.e.*, an aminosaccharide or polyaminosaccharide. For example, reaction with muramic acid is expected to proceed as follows.



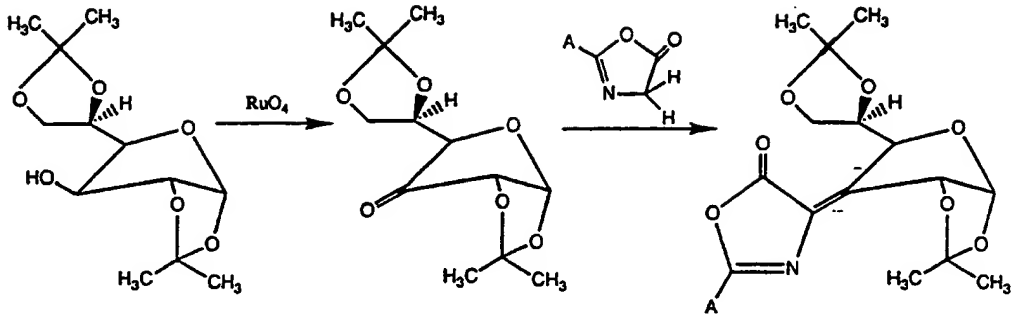
Similar treatment which is shown below, of the structurally interesting ambecide paromomycin, with 1 to 5 equivalents of a tailored oxazolone is expected to produce a series of novel structures in which a branched tetrasaccharide scaffold supports peptidomimetic structures derived from oxazolones in a geometrically defined manner.

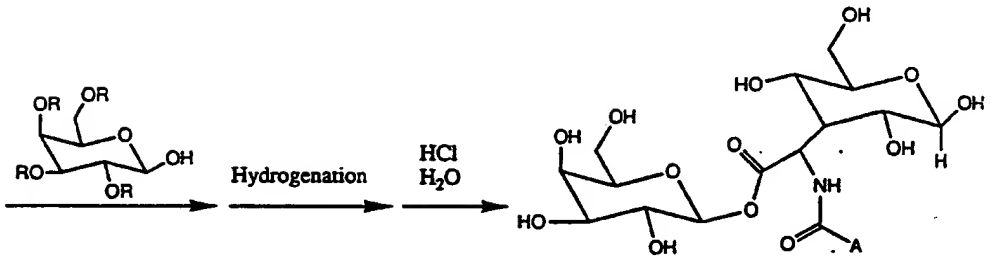


Paromomycin

(3) Use of oxazolone-derived structures as replacements of glycosidic linkages.

Selective blocking of all but one hydroxyl in a sugar allows the selective oxidation of the hydroxyl to the carbonyl-derivative, which can then be used in an aldol-type condensation reaction with a methylene oxazolone to produce an alkene oxazolone; this can then be ring-opened, by *e.g.*, the anomeric hydroxyl of a sugar to give a novel saccharide after deprotection.





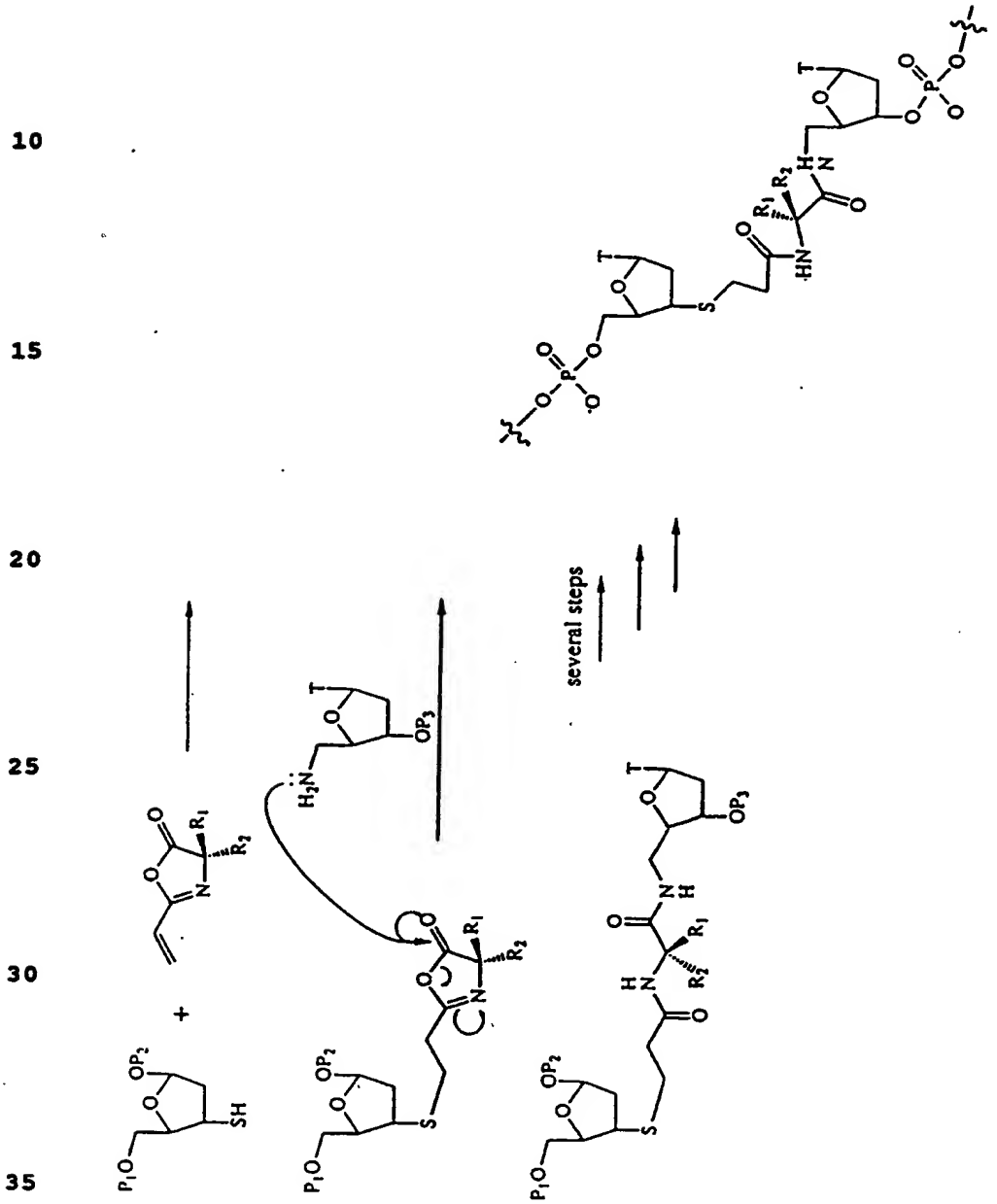
OLIGONUCLEOTIDES

4.2.7 Design and Synthesis of Oxazolone-Derived Oligonucleotide Mimetics

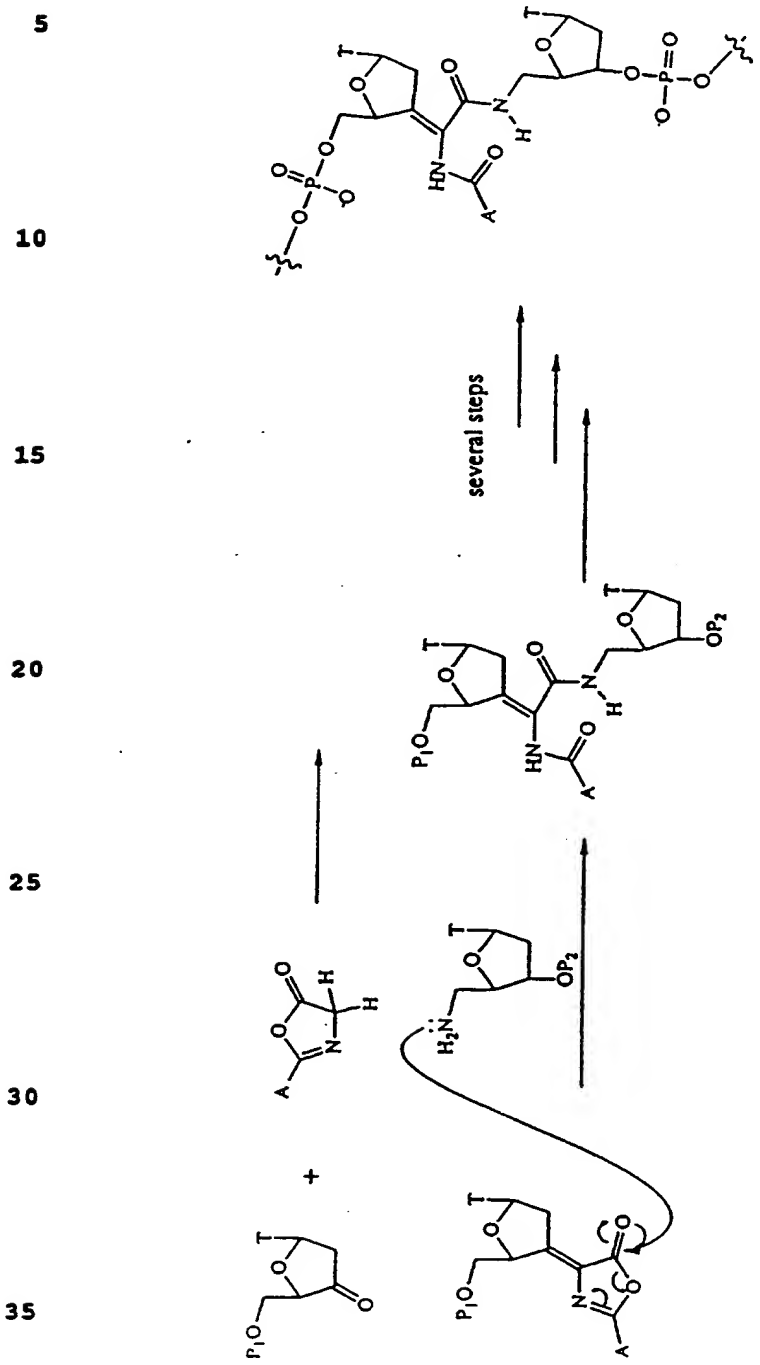
The art of nucleotide and oligonucleotide synthesis has provided a great variety of suitably blocked and activated furanoses and other intermediates which are expected to be very useful in the construction of oxazolone-derived mimetics (Comprehensive Organic Chemistry, Sir Derek Barton, Chairman of Editorial Board, Vol. 5, E. Haslam, Editor, pp. 23-176).

A great variety of nucleotide and oligonucleotide structural motifs incorporating oxazolone-derived structures are contemplated including, but not limited to, the following.

(1) For the synthesis of oligonucleotides containing peptidic oxazolone-derived linkers in place of the phosphate diester groupings found in native oligonucleotides, the following approach is one of many that is expected to be useful.

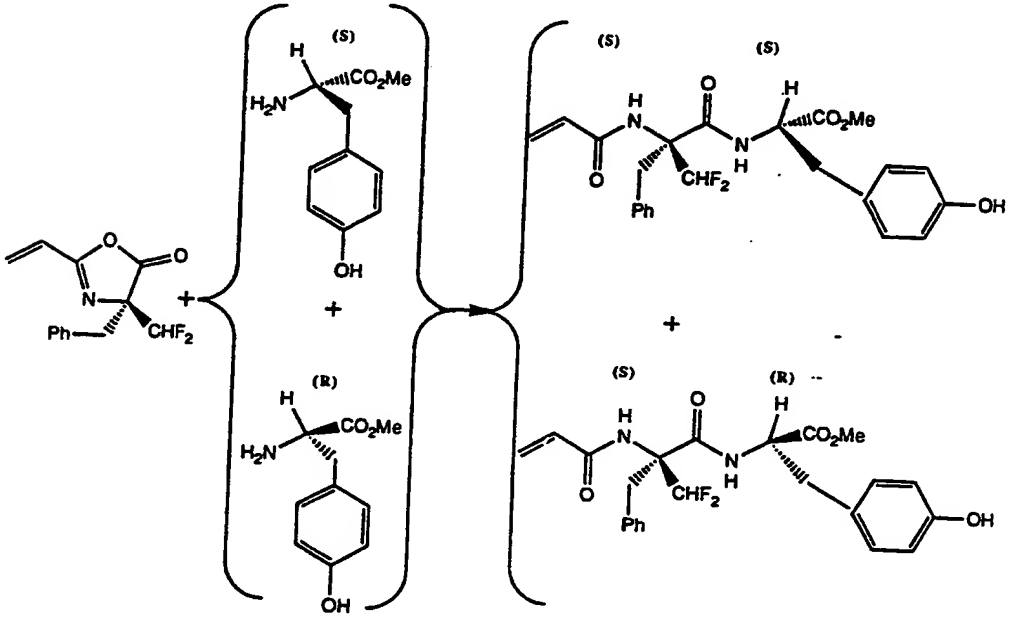


(2) For the synthesis of structures in which an oxazolone-derived grouping is used to link complex oligonucleotide-derived units, an approach such as the following is expected to be useful.



5. Example: Characterization of the Enantiometric Purity of Oxfenacine

This example teaches the use of the ring opening reaction of the pure chiral isomer azalactone (S)-(-)-4-difluoromethyl-4-benzyl-2-vinyl-5-oxazolone (1) with racemic mixtures of the methyl esters of (R)- and (S)-p-hydroxyphenylglycine to form the diastereomeric conjugates (2) and (3), as shown:



These diastereomers can be separated by standard HPLC methods on normal-phase silica to quantitatively assay the enantiomeric composition of th starting p-hydroxyphenylglycines from which the esters are produced.

The (S)-isomer of p-hydr xyphenylglycin

(oxfenacine) is an effective therapeutic agent for promoting the oxidation of carbohydrates when this process is depressed by high fatty acid utilization levels (such as occurs in ischemic heart disease), and is also an important chiral intermediate in the production of penicillin, amoxicillin and several other semisynthetic antibiotics, including the cephalosporins. Oxfenacine is prone to racemization, and the assay for chiral purity described in this example therefore represents a useful development and quality-control tool.

6. Example: Resolution of Racemic *p*-Hydroxyphenyl Glycine Esterification of p-hydroxyphenyl glycine

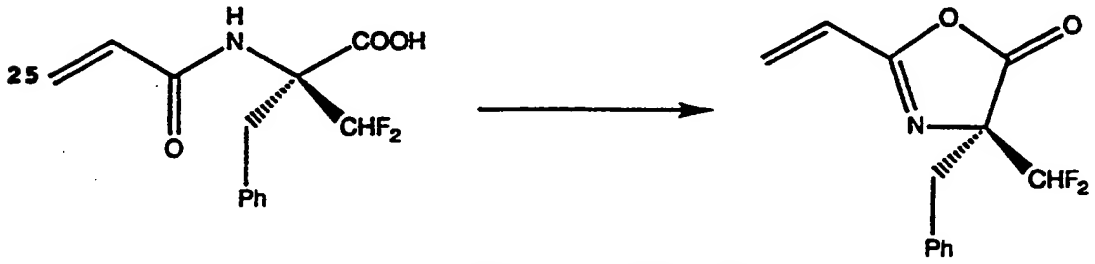
0.3 g (0.2 ml) thionyl chloride was added dropwise to 5 ml of a stirred solution of 0.4 g of the stereoisomeric mixture of 4-hydroxyphenylglycine enantiomers to be characterized in methanol and the temperature of the mixture kept between 10 and 20°C with ice cooling. The reaction was allowed to proceed at room temperature for 1 hour. The methanol was then removed at room temperature under aspirator vacuum (10 torr) on a rotary evaporator and a solid was obtained. This solid was dissolved in 10 ml of deionized water and the pH adjusted to 9.2 with 0.88 M ammonium hydroxide. The solution was then stirred for 1 hour at 10°C and the precipitated solid ester mixture was filtered off, washed with deionized water and dried at 45°C under vacuum to give 0.41 g of product (94%).

Ring-Opening Addition. 0.181 g (0.001 mol) of the esterified 4-hydroxyphenylglycine prepared as outlined above was dissolved in 10 ml of peroxide-free dry dioxane. To this mixture was added 0.251 g (0.001 mol) of (S)-4-difluoromethyl-4-benzyl-2-vinyl-5-oxazolone, and the resulting s lution heated at reflux for 2 hours. The dioxane was removed by rotary evaporation and 0.43 g

(100%) of the pale yellow solid amide residue was isolated.

HPLC Analysis. A solution of the diastereomeric amides was prepared in methylene chloride at a concentration of 7 mg/ml. This solution was injected into a DuPont Model 830 liquid chromatograph equipped with a detector set at 254 nm using a 20 μl loop valve injection system. The sample was chromatographed on a 25 cm x 0.4 cm stainless steel HPLC column packed with 5μ Spherisorb S5W silica gel using a 98/1/1 cyclohexane/n-butanol/isopropanol mobile phase at a flow rate of 0.9 ml/min. The enantiomeric amide conjugates were then quantitated using a calibration curve generated with a series of synthetic mixtures containing varying ratios of the two pure enantiomers. The pure L-isomer was purchased from Schweizerhall Inc. The pure D-isomer was prepared from the commercially available D,L-racemate obtained from MTM Research Chemicals/Lancaster Synthesis Inc. by the method of Clark, Phillips and Steer (J. Chem. Soc., Perkins Trans. I at 475 [1976]).

(S)-4-difluoromethyl, 4-benzyl-2-vinyl-5-oxazolone



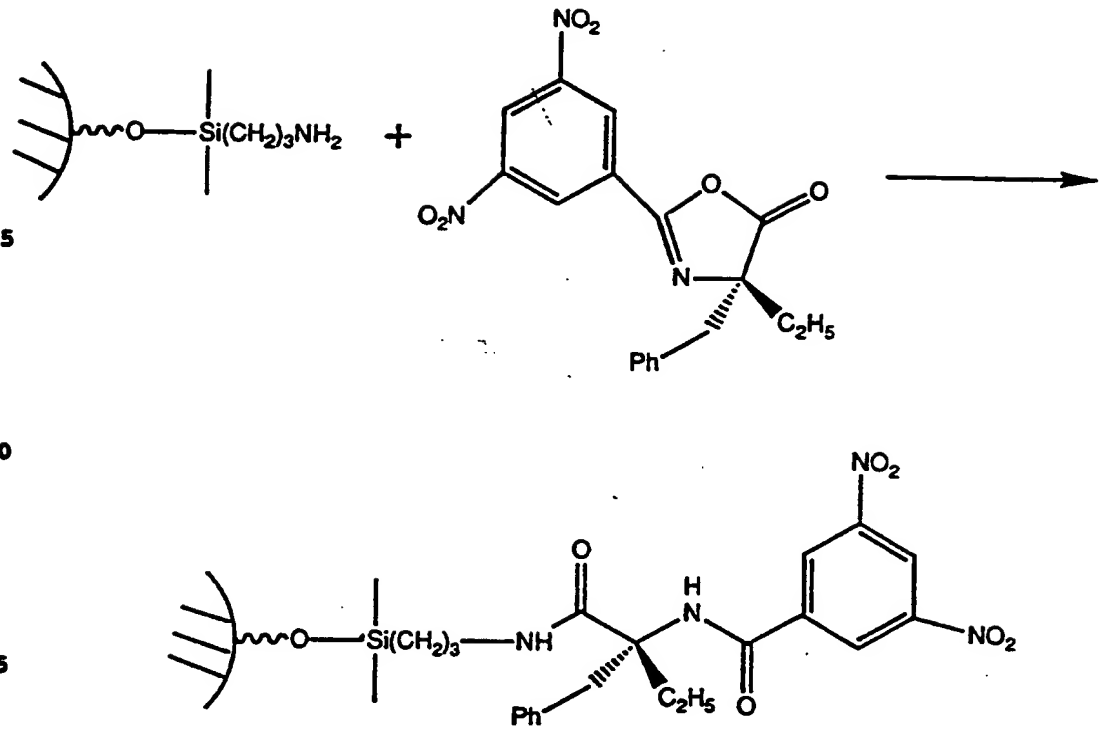
5.43 g (0.05 mol) of ethyl chloroformate was added with stirring to 13.46 g (0.05 mol) of N-acryloyl-(S)-2-difluoromethyl phenylalanine in 75 ml of dry acetone at room temperature. 7.0 ml (0.05 mol) of triethylamine were then added dropwise over a period of 10 min., and the mixture was stirred at room temperature until gas evolution ceased (1.5 h urs). The

triethylamine hydrochloride was removed by filtration, the cake was slurried in 25 ml of acetone and refiltered. The combined filtrates were concentrated to 50 ml on a rotary evaporator, refiltered, cooled to -30°C and the crystallized product was collected by filtration and dried *in vacuo* to give 10.05 g (80%) of (S)-4-difluoromethyl-4-benzyl-2-vinyl azlactone. NMR ($CDCl_3$); CH_2 = CH - chemical shifts, splitting pattern in 6 ppm region and integration ratios diagnostic for structure. FTIR (mull) strong azlactone CO band at 1820 cm^{-1}.

N-Acryloyl-(S)-2-difluoromethyl phenylalanine.

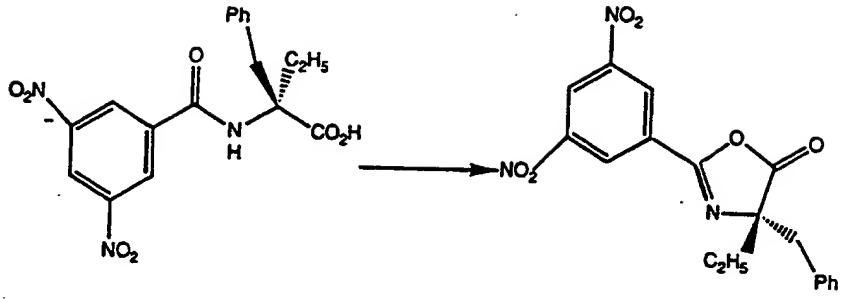
21.5 g (0.1 mol) (S)-2-difluoromethyl phenylalanine, prepared using the method described by Kolb and Barth (Liebigs Ann. Chem. 1668 (1983)), was added with stirring to a solution of 8.0 g (0.2 mol) of sodium hydroxide in 100 ml water and stirred at this temperature until complete solubilization was achieved. 9.05 g (0.1 mol) acryloyl chloride was then added dropwise with stirring, keeping the temperature at 10-15°C with external cooling. After addition was complete, stirring was continued for 30 min. To this solution 10.3 ml (0.125 mol) of concentrated hydrochloric acid was added over a 10-min. period, keeping the temperature at 15°C. After addition was complete, the reaction mixture was stirred an additional 30 min., cooled to 0°C, and the solid product was collected by filtration, washed well with ice water and pressed firmly with a rubber dam. The resulting wet cake was recrystallized from ethanol/water to yield 18.8 g (70%) of N-acryloyl-(S)-2-difluoromethyl phenylalanine. NMR ($CDCl_3$): chemical shifts, CH_2 = CH - splitting pattern and integration ratios diagnostic for structure

7. Example: Preparati n of Chiral Chromatographic Stationary Phase Ring Opening Formation of Conjugate with Aminopropyl Silica



5.0 g of aminopropyl-functionalized silica was slurried in 100 ml benzene in a three-necked flask equipped with a stirrer, a heating bath, a reflux condenser and a Dean-Stark trap. The mixture heated to reflux and the water removed azeotropically. 3.69 g (0.01 mol) of (S)-4-ethyl,4-benzyl-2-(3',5'-dinitrophenyl)-5-oxazolone was added and the mixture was heated at reflux for 3 hours. The mixture was subsequently cooled, and the silica collected on a Buechner filter and washed with 50 ml benzene. The wet cake was reslurried in 100 ml methanol and refiltered a total of four times. The resulting product was dried in a vacuum oven set for 30" and 60°C to yield 4.87 g functionalized silica. The bonded phase was packed into a 25 cm x 0.46 cm stainless-steel HPLC column from methanol, and successfully used to separate a series of mandelic acid derivatives using standard conditions.

(S)-4-ethyl,4-benzyl-2-(3',5'-dinitrophenyl)-5-oxazolone



1.09 g (0.01 mol) of ethyl chloroformate was added with stirring to 3.87 g (0.01 mol) N-3,5-dinitrobenzoyl-(S)-2-ethyl phenylalanine in 75 ml dry acetone at room temperature. 1.4 ml (0.01 mol) of triethylamine was added dropwise over a 10-min. period and the mixture was stirred at room temperature until gas evolution ceased (1.5 hours). The triethylamine hydrochloride was removed by filtration and the cake was slurried with 25 ml acetone and refiltered. The combined filtrates were concentrated to 50 ml on a rotary evaporator, refiltered, cooled to -30°C and the crystallized roduct was collected by filtration and dried *in vacuo* to yield 2.88 g (78%) of (S)-4-ethyl-4-benzyl-2-(3',5'-dinitrophenyl)azlactone. NMR ($CDCl_3$): Frequencies and integration ratios diagnostic for structure. FTIR: strong azlactone band at ca. 1820 cm^{-1}.

N-3,5-dinitrobenzloyl-(S)-2-ethylphenylalanine

19.3 g (0.1 mol) of (S)-2-ethylphenylalanine, prepared from (S)-phenylalanine and ethyl iodide using the method described by Zydowsky, de Lara and Spanton (55 J. Org. Chem. 5437 (1990)) was added with stirring to a solution of 8 g (0.2 mol) sodium hydroxide in 100 ml water and cooled to about 10°C. The mixture was then stirr d at this temperature until complete solubilization was achieved. 23.1 g (0.1 mol) 3,5-dinitrobenzoyl

chloride was then added dropwise with stirring, keeping the temperature at 10-15°C with external cooling. After this addition was complete, stirring was continued for 30 min. To this solution was added 10.3 ml (1.25 mol) of concentrated HCl over a 10 min. period, again keeping the temperature at 15°C. During this addition a white solid formed. After the addition was complete, the reaction mixture was stirred for an additional 30 min., cooled to 0°C and the white solid was collected by filtration, washed well with ice water and pressed firmly with a rubber dam. The resulting wet cake was recrystallized from ethanol/water and dried in a vacuum oven set for 30" at 60°C to yield 27.1 g (70%) N-3,5-dinitrobenzoyl-(S)-2-ethyl phenylalanine.

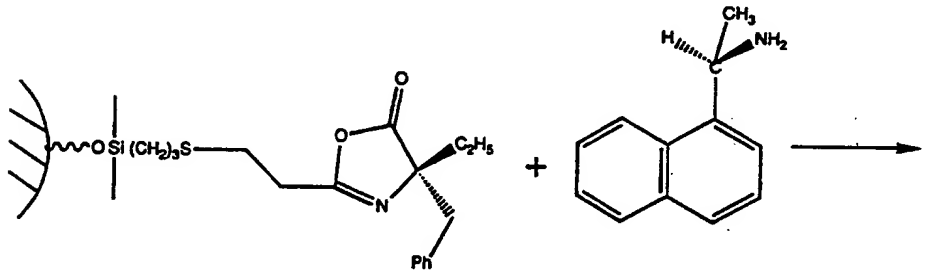
Preparation of Aminopropyl-Functionalized Silica.

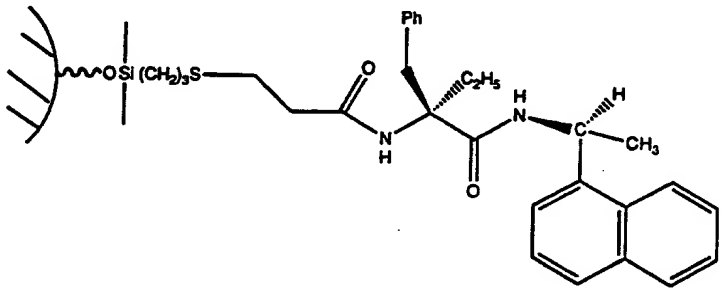
200 g 015M Spherosil (IBF Corporation) was added to 500 ml toluene in a one-liter three-necked round-bottomed flask equipped with a Teflon paddle stirrer, a thermometer and a vertical condenser set up with a Dean-Stark trap through a claisen adaptor. The slurry was stirred, heated to a bath temperature of 140°C and the water azeotropically removed by distillation and collected in the Dean-Stark trap. The loss in toluene volume was measured and compensated for by the addition of incremental dry toluene. 125.0 g 3-aminopropyltrimethoxysilane was added carefully through a funnel and the mixture stirred and refluxed for 3 hours with the bath temperature set at 140°C. The reaction mixture was cooled to about 40°C and the resulting functionalized silica collected on a Buechner filter. The silica was then washed twice with 50 ml toluene, sucked dry, reslurried in 250 ml toluene, refiltered, reslurried in 250 ml methanol and refiltered a total of three times. The resulting methanol wet cake was dried

in a vacuum oven set for 30" at 60°C to yield 196.4 g aminopropyl silica.

8. Example: Ring-Opening Conjugation of (S)-1-(1-naphthyl)ethylamine With The Michael-Addition Product Of Aminomercapto-Functionalized Silica And (S)-4-Ethyl-4-benzyl-2-acryloyl-5-oxazolone To Produce A Chiral Chromatographic Stationary Phase

Formation Of Conjugate With (S)-(1)-(1-naphthyl)-ethylamine

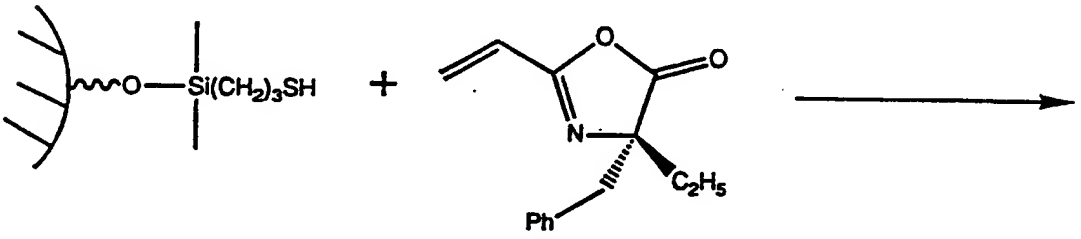


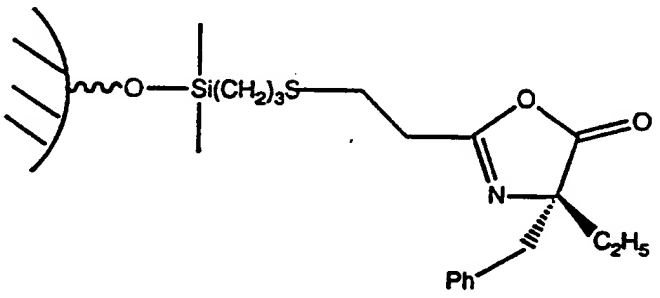


10.0 g (S)-4-ethyl-4-benzyl-2-(ethylthiopropyl silica)-5-oxazolone was slurried in 100 ml benzene in a three-necked flask equipped with a stirrer, a heating bath, a reflux condenser and a Dean-Stark trap. The mixture was heated to reflux and the water was removed azeotropically. 3.42 g (0.02 mol) (S)-(-)- (1-naphthyl)ethylamine was added and the mixture was heated at reflux for 6 hours. The mixture was then co l d, the silica collected on a Buechner filter and washed with 100 ml benzene. The w t cake was reslurried in 100 ml

methanol and refiltered a total of four times. The product was dried in a vacuum oven set for 30" and 60°C to give 9.72 g functionalized silica. The bonded phase was packed into a 25 cm x 0.46 cm stainless-steel HPLC column from methanol and successfully used to separate a series of π-acceptor amine derivatives using standard conditions described in the Chromatography Catalog distributed by Regis Chemical, Morton Grove, Ill. 60053 (e.g., the 3,5-dinitro benzoyl derivatives of racemic 2-amino-1-butanol + alpha methyl benzye amine).

Michael Addition by Mercaptopropyl Silica





20 g mercaptopropyl silica was added to 200 ml benzene in a 500 ml three-necked round-bottomed flask equipped with a Teflon paddle stirrer, a thermometer and a vertical condenser set up with a Dean-Stark trap through a claisen adaptor. The slurry was stirred, heated to a bath temperature of 140°C and the water azeotropically rem ved by distillation and collected in the Dean-Stark trap. The 1 ss in benzene volume was

measured and compensated for by the addition of incremental dry benzene. 6.88 g (0.03 mol) of (S)-4-ethyl,4-benzyl-2-vinyl-5-oxazolone was added and the mixture was stirred and refluxed for 16 hours. The reaction mixture was then cooled to about 40°C. The resulting functionalized silica was collected on a Buechner filter, washed with 50 ml benzene, sucked dry, reslurried in 100 ml of methanol and refiltered a total of four time. The resulting methanol wet cake was dried in a vacuum oven set for 30" at 60°C to yield 19.45 g oxazolone-functionalized silica.

(S)-4-ethyl-4'-benzyl-2-acryloyl-5-oxazolone.

10.9 g (0.1 mol) of ethyl chloroformate was added with stirring to 24.7 g (0.1 mol) of N-acryloyl-(S)-2-ethyl phenylalanine in 250 ml dry acetone at room temperature. 14 ml (0.1 mol) of triethylamine was added dropwise over a 10-min. period and the mixture was stirred at room temperature until gas evolution ceased (1.5 hours). The triethylamine hydrochloride was removed by filtration and the cake was slurried with 50 ml of acetone and refiltered. The combined filtrates were concentrated to 150 ml on a rotary evaporator, refiltered, cooled to -30°C and the crystallized product was collected by filtration and dried *in vacuo* to yield 19.5 g (85%) (S)-4-ethyl-4-benzyl-2-vinyl-5-azlactone. NMR 9CDCl): chemical shifts, CH_2 = CH - splitting pattern in 6 ppm region + integration ratios diagnostic for structure. FTIR + (mull): strong azlactone CO band in 1820 cm^{-1} region.

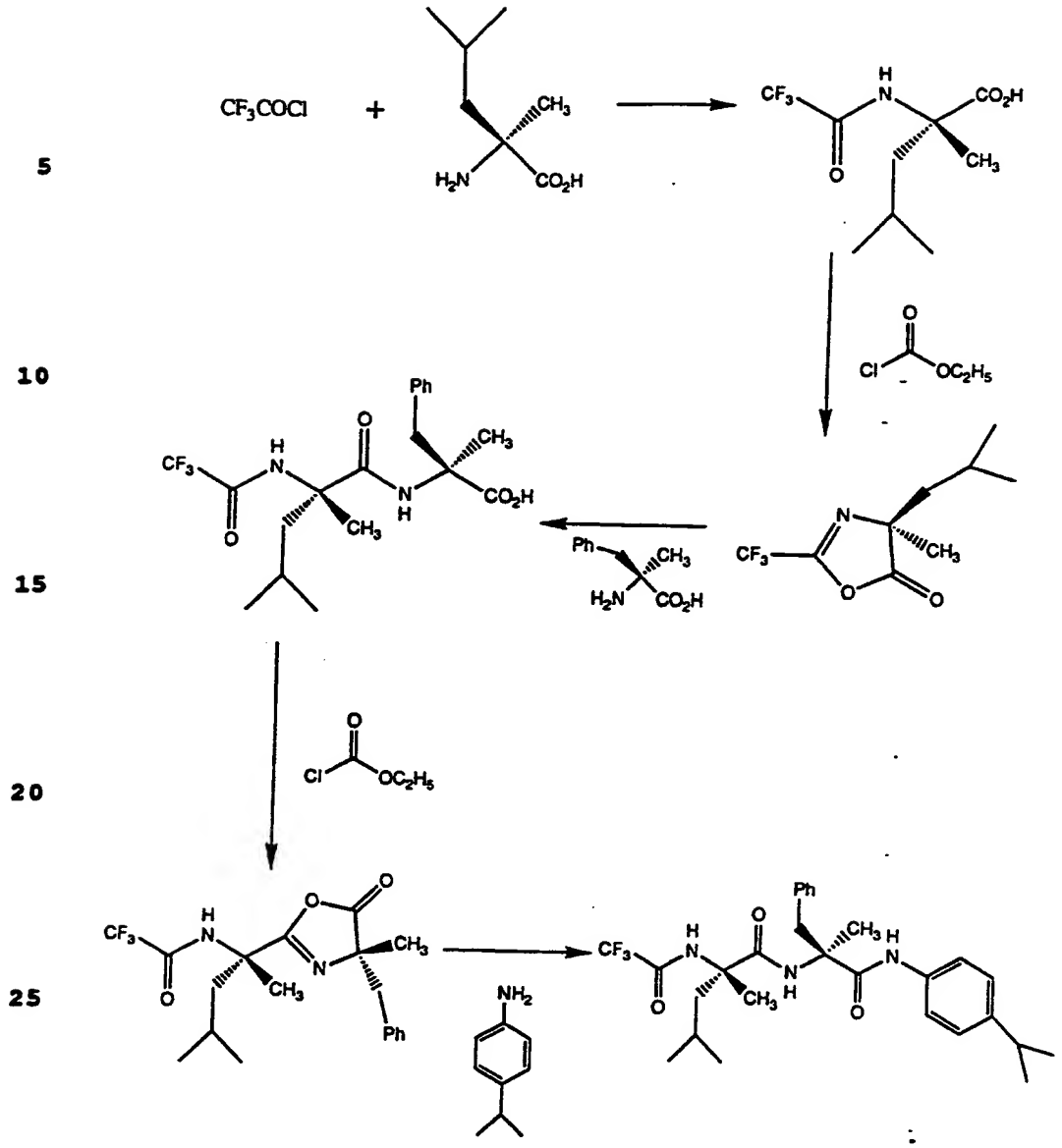
Preparation of Mercaptopropyl-Functionalized Silica. 200 g of 10μ (80A) Exsil silica (Exnere Ltd.) was added to 500 ml toluene in a one-liter three-necked round-bottomed flask equipped with a Tefl n paddle stirrer, a thermometer and a vertical condenser set up with a Dean-

Stark trap through a claisen adaptor. The slurry was stirred, heated to a bath temperature of 140°C and the water was azeotropically removed by distillation and collected in the Dean-Stark trap. The loss in toluene volume was measured and compensated for by the addition of incremental dry toluene. 110.0 g of 3-mercaptopropyltrimethoxysilane was added carefully through a funnel and the mixture was stirred and refluxed for 3 hours with the bath temperature set at 140°C. The reaction mixture was then cooled to about 40°C. The resulting functionalized silica was collected on a Buechner filter, washed twice with 50 ml toluene, sucked dry, reslurried in 250 ml toluene, refiltered, reslurried in 250 ml methanol and refiltered a total of three times. The resulting methanol wet cake was dried in a vacuum oven set for 30" at 60°C to yield 196.4 g of mercaptopropyl silica.

Chiral azlactone conjugates may similarly be produced using a variety of azlactone derivatives containing at the 2-position other groups capable of undergoing addition (and sequential ring-opening) reactions. Examples of these groups include hydroxyalkyl, haloalkyl and oxirane groups.

9. Example: Synthesis of a Mimetic of Known Human Elastase Inhibitor

This example teaches the synthesis of a competitive inhibitor for human elastase based on the structure of known N-trifluoroacetyl dipeptide analide inhibitors - see, e.g., 107 Eur. J. Biochem. 423 (1980); 162 J. Mol. Biol. 645 (1982) and references cited therein.



N-trifluoroacetyl-(S)-2-methyl leucyl-(S)-2-ethylphenylalanyl-p-isopropylanlide.

0.135 g (0.001 mol) 4-isopropyl analine is dissolved in the minimum amount of an appropriate solvent, such as acetonitrile, and 0.384 g (0.001 mol) of 2-(N-trifluoroacetyl-(S)-2-methyl leucyl)-(S)-4-methyl-4-benzyl-5-oxazolone dissolved in the minimum amount of the same solvent is added gradually to the stirred solution with cooling. Following addition, the reaction mixture is allowed to come to room remperature and is stirred at room temperature for 36 hours. The solvent is then removed *in vacuo* to yield the solid N-trifluoroacetyl-(S)-2-methyl-leucyl-(S)-2-ethylphenylalanyl analide, useful as a competitive inhibitor of human elastase in essentially quantitative yield.

2-(N-trifluoroacetyl-(S)-2-methylleucyl)-(S)-4-methyl-4-benzyl-5-oxazolone.

4.1 g (0.01 mol) N-trifluoroacetyl-(S)-2-methylleucyl-(S)-2-methylphenylalanine lithium salt is slurried in 50 ml of an appropriate solvent, such as dry benzene, in a three-necked round-bottomed flask equipped with a stirrer, heating bath, claisen head, downward condenser, thermometer and dropping funnel. The system is heated to 65°C, and 1.09 g (0.01 mol) of ethyl chloroformate dissolved in 10 ml dry benzene is added over a 10-min. period. Addition is accompanied by the vigorous evolution of gas and the distillation of a benzene/ethanol azeotrope. Following the completion of the addition, heating is continued for 30 min. The heating bath is then removed and the slurry is stirred for an additional 15 min. The precipitated lithium chloride is carefully removed by filtration and the cake is triturated with benzene and refiltered. The combined filtrates are stripped using a pot temperature of 40°C to yield 3.50 g (90%) of crude oxazolone. The product was purified by recrystallization from acetone at -30°C. FTIR (mull): Strong azlactone CO band in 1820 cm^{-1} regi n.

N-trifluoracetyl-(S)-2-methylleucyl-(S)-2-methylphenylalanine.

2.23 g (0.01 mol) 2-trifluoroacetyl-(S)-4-methyl-4-isobutyl-5-oxazolone is dissolved with stirring in the minimum amount of an appropriate solvent, such as acetonitrile, and 1.85 g (0.01 mol) of the lithium salt of (S)-2-methyl phenylalanine in the minimum amount of the same solvent is added gradually, and with cooling. This salt is obtained by treatment of (S)-2-methylphenylalanine (produced from (S)-phenylalanine and methyl iodide using the method of Zydoski et al., 55 J. Org. Chem. 5437 (1990)) with one equivalent of LiOH in an appropriate solvent, such as ethanol, followed by removal of the solvent *in vacuo*. After addition of the lithium salt, the reaction mixture is allowed to warm to room temperature and is stirred at room temperature for 36 hours. The solvent is then removed *in vacuo* to yield the solid N-trifluoroacetyl-(S)-2-methylleucyl-(S)-2-methylphenylalanine lithium salt in essentially quantitative yield.

2-trifluoroacetyl-(S)-4-methyl-4-isopropyl-5-oxazolone.

12.05 g (0.05 mol) of N-trifluoroacetyl-(S)-2-methyl-leucine was stirred at room temperature in 100 ml dry acetone and 5.43 g (0.05 mol) ethyl chloroformate was added. 7.0 ml (0.05 mol) of triethylamine was added dropwise over a period of 10 min. and the mixture was stirred at room temperature until gas evolution ceased (1.5 hours). The triethylamine hydrochloride was removed by filtration and the cake was slurried with 25 ml of acetone and refiltered. The combined filtrates were concentrated to 75 ml on a rotary evaporator, refiltered, cooled to -30°C and the crystallized product was collected by filtration and dried *in vacuo* to yield 10.6 g (88%) of (S)-4-methyl-4-isobutyl-2-trifluoroacetyl-5-

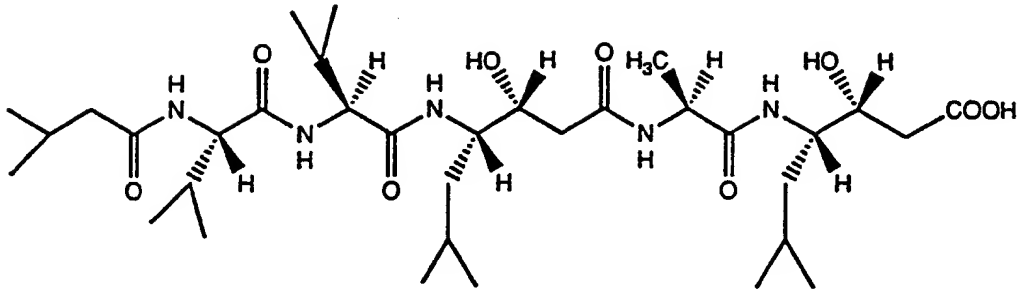
oxazolone. FTIR (mull): strong azlactone CO band in 1820 cm^{-1} region.

N-trifluoroacetyl-(S)-2-methyl-leucine.

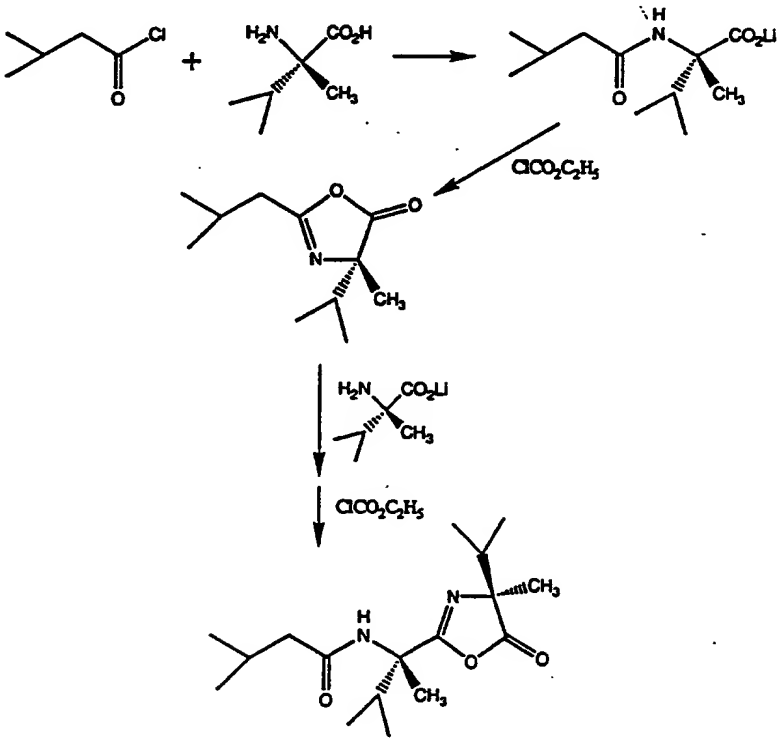
14.5 g (0.1 mol) of (S)-2-methyl-leucine, prepared from D,L-leucine methyl ester hydrochloride using the method of Kolb and Barth (Liebig's Ann. Chem. at 1668 (1983)) was added with stirring to a solution of 8 g (0.2 mol) of sodium hydroxide in 20 ml water, cooled to 10°C, and the mixture stirred at this temperature until complete solubilization was achieved. 13.25 g (0.1 mol) trifluoroacetyl chloride was then added dropwise with stirring, keeping the temperature at 10°C with external cooling. After the addition was complete, stirring was continued for 30 min. To this solution was added, over a 10-min. period, 10.3 ml (0.125 mol) of concentrated hydrochloric acid, again keeping the temperature at 15°C During the addition, a white solid formed. After the addition was complete, the reaction mixture was stirred for an additional 30 min. and cooled to 0°C. The white solid was collected by filtration, washed well with ice water and pressed firmly with a rubber dam. The resulting wet cake was recrystallized from ethanol/water and dried *in vacuo* to give 17.4 g (72%) of N-trifluoroacetyl-(S)-2-methyl-leucine which was used directly in the following step in the sequence (above).

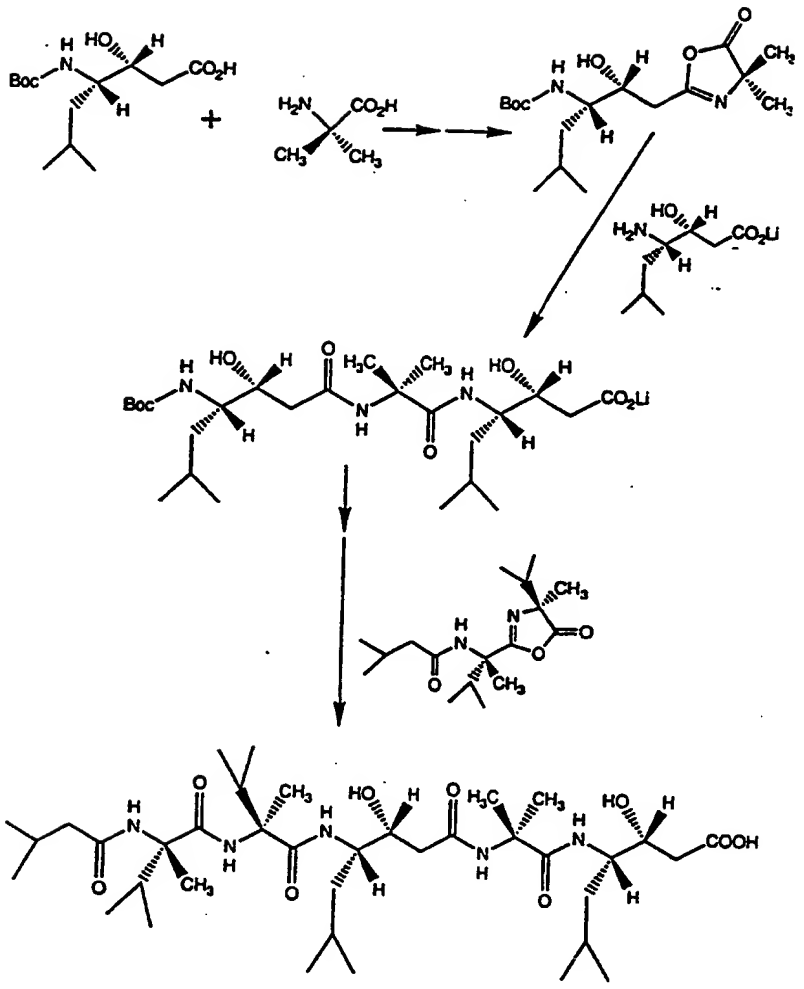
11. Example: Synthesis of a Pepstatin Mimetic

This example teaches the synthesis of an oxazolone-derived mimetic of the known aspartyl protease inhibitor, pepstatin, which has the structure shown:



This mimetic is useful as a competitive inhibitor for proteases inhibited by pepstatin.





N-isovaleryl-(S)-2-methylvaleryl-(3S,4S)-statyl-(S)-2-methyl-alanyl-(3S,4S)-statine.

The Boc-protected lithium salt prepared as described below simultaneously converted to the acid form and deprotected by treatment with acid under standard deprotection conditions. 5.17 g (0.01 mol) of N-isovaleryl-(S)-2-methy derivative added to 100 ml dry acetonitrile, stirred at room temperature and 3.17 g (0.01 mol) of the valyl-(S)-4-methyl-4-isopropyl-5-oxazolone was added with cooling. Once addition was complete, the mixture was heated to reflux and held at reflux for 1 hour. The solvent then stripped *in vacuo* to give a quantitative yield of N-isovaleryl-(S)-2-methylvalyl-(3S,4S)-statyl-(S)-2-methylalanyl-(3S,4S)-statine, useful as a pepstatin-mimetic competitive inhibitor for aspartyl proteases which are inhibited by pepstatin (see, 23 J. Med. Chem. 27 (1980) and references cited therein). NMR (d_6 DMSO): chemical shifts, integrations and D_2O exchange experiments diagnostic for structure.

N-Boc-(3S,4S)-statyl-(S)-2-methylalanyl-(3S,4S)-statine lithium salt.

6.84 g (0.02 mol) of the Boc-protected oxazolone prepared below stirred in 100 ml of dry acetonitrile at room temperature and 3.62 g (0.02 mol) of the lithium salt of (3S,4S)-statine, prepared from statine using the method outlined below, was added with cooling. Once addition was complete, the mixture was heated to reflux and held at reflux for 1 hour. The solvent was then stripped *in vacuo* to give a quantitative yield of N-Boc-(3S,4S)-statyl-(S)-2-methylalanyl-(3S,4S)-statine lithium salt.

Boc-protected (3S,4S)-statine, [(3S,4S)-4-amino-3-hydroxy-6- methylheptanoic acid] was produced from the commercially available amino acid, coupled with

2-methylalanine using standard peptide synthesis methods and converted to the lithium salt using the method described below. 18.30 g (0.05 mol) of this derivative was stirred in 150 ml dry acetonitrile at room temperature, 5.45 g (0.05 mol) of ethyl chloroformate and 7.0 ml (0.05 mol) of triethylamine were sequentially added with stirring and the mixture was stirred at room temperature until gas evolution ceased (1.5 hours). The mixture was then stripped to dryness on a rotary evaporator, the residue was triturated with 100 ml of benzene, filtered to remove salts, and the filtrate was again stripped on a rotary evaporator to yield 16.4 g (96%) of crude 2-BOC-(3S,4S)-statyl-4,4-dimethyl-5-oxazolone. Analytically pure material was obtained by recrystallization from acetone at -30°C. NMR ($CDCl_3$) - chemical shifts and splitting patterns diagnostic for structure. FTIR (mull): shows a strong azlactone CO band in the 1820 cm^{-1} region.

N-isovaleryl-(S)-2-methylvalyl-(S)-4-methyl-4-isopropyl-5-oxazolone.

13.46 g (0.04 mol) of 2-isovaleryl-(S)-2-methylvalyl-(S)-2- methyl valine lithium salt, as prepared below, was stirred in 150 ml of dry acetonitrile at room temperature. 4.36 g (0.04 mol) of ethyl chloroformate and 5.6 ml (0.04 mol) of triethylamine were then sequentially added with stirring, and the mixture was stirred at room temperature until gas evolution ceased (1.5 hours). The mixture was then stripped to dryness on a rotary evaporator, the residue was triturated with 100 ml benzene, filtered to remove salts, and the filtrate was again stripped on a rotary evaporator to yield 12 g (96%) of crude N-isovaleryl-(S)-2-methylvalyl-(S)-4-methyl-4-isopropyl-5-oxazolone. Analytically pure material was obtained by recrystallization fr m acetone at -30°C. NMR ($CDCl_3$):

chemical shifts and splitting patterns diagnostic f r structure. FTIR (mull): shows strong azlactone CO band in the 1820 cm^{-1} region.

N-isovaleryl-(S)-2-methylvalyl-(S)-2-methyl valine lithium salt.

6.85 g (0.05 mol) of (S)-2-methylvaline lithium salt, prepared from (S)-methyl valine by the method described below, was stirred in 150 ml dry acetonitrile at room temperature and 9.93 g (0.05 mol) of the oxazolone prepared below was added portionwise with cooling. Once addition was complete, the mixture was heated to reflux and held at reflux for 1 hour. The solvent was then stripped *in vacuo* to give a 98% yield of N-isovaleryl-(S)-2-methylvalyl-(S)-2-methyl valine lithium salt. This salt was used directly in the next step (above).

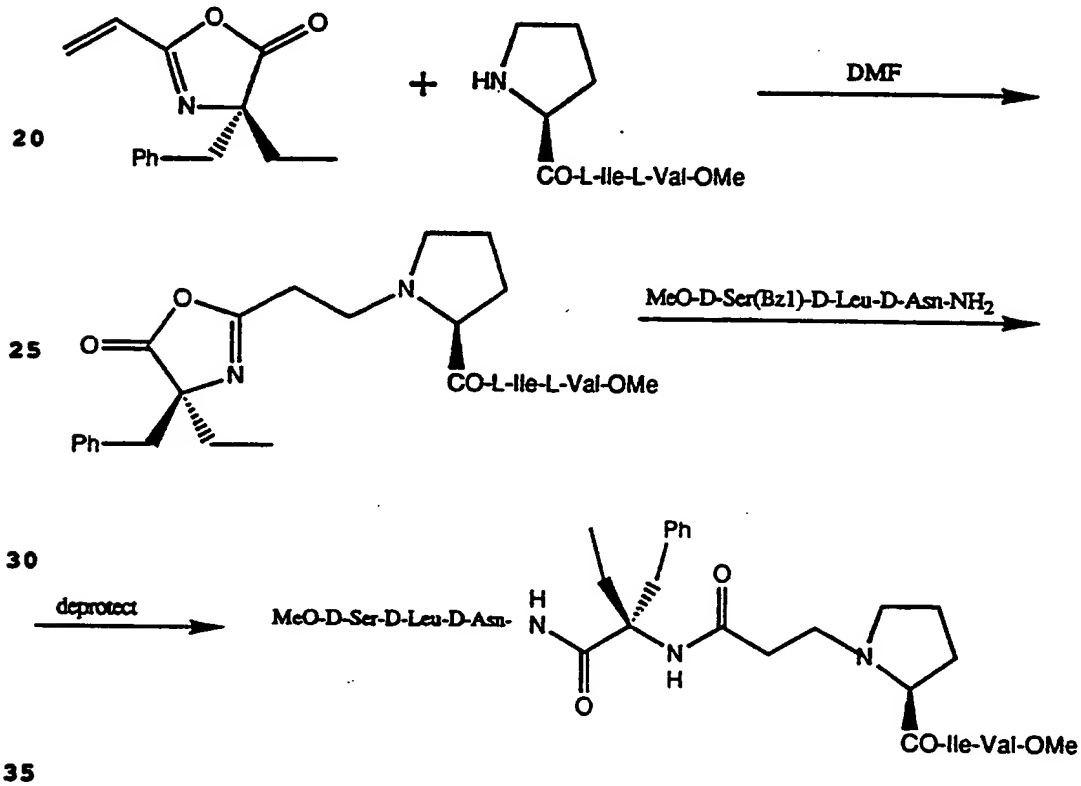
2-isovaleryl-(S)-4-methyl-4-isopropyl-5-oxazolone.

2-(S)-methylvaline was prepared from (S)-valine by the method described by Kolbe and Barth (Liebigs Ann. Chem. at 1668 (1983)), and was acylated with isovaleryl chloride using standard acylation methods to produce N-isovaleryl-(S)-methylvaline, this was subsequently treated with one equivalent of LiOH in ethanol, followed by removal of the solvent *in vacuo* to yield the N-isovaleryl-(S)-methylvaline lithium salt. 22.3 g (0.1 mol) of this Li salt was stirred in 150 ml of dry acetonitrile at room temperature, 10.9 g (0.01 mol) of ethyl chloroformate and 14 ml (0.1 mol) of triethylamine were sequentially added with stirring, and the mixture was stirred at room temperature until gas evolution ceased (1.5 hours). The mixture was then stripped to dryness on a rotary evaporator, the residue was triturated with 150 ml benzene, filtered to remove salts and the filtrate was again stripped on a rotary

evaporator to yield 17.4 g (85%) of crude 2-is valeryl-(S)-4-methyl-4-isopropyl-5-oxazolone. Analytically pure material was obtained by re-crystallization from acetone at -30°C. FTIR (mull): shows a strong azlactone CO band in the 1820 cm^{-1} region. NMR ($CDCl_3$): chemical shifts and splitting patterns diagnostic for structure.

12. Example: Synthesis of a Mimetic Inhibitor of the HIV Protease

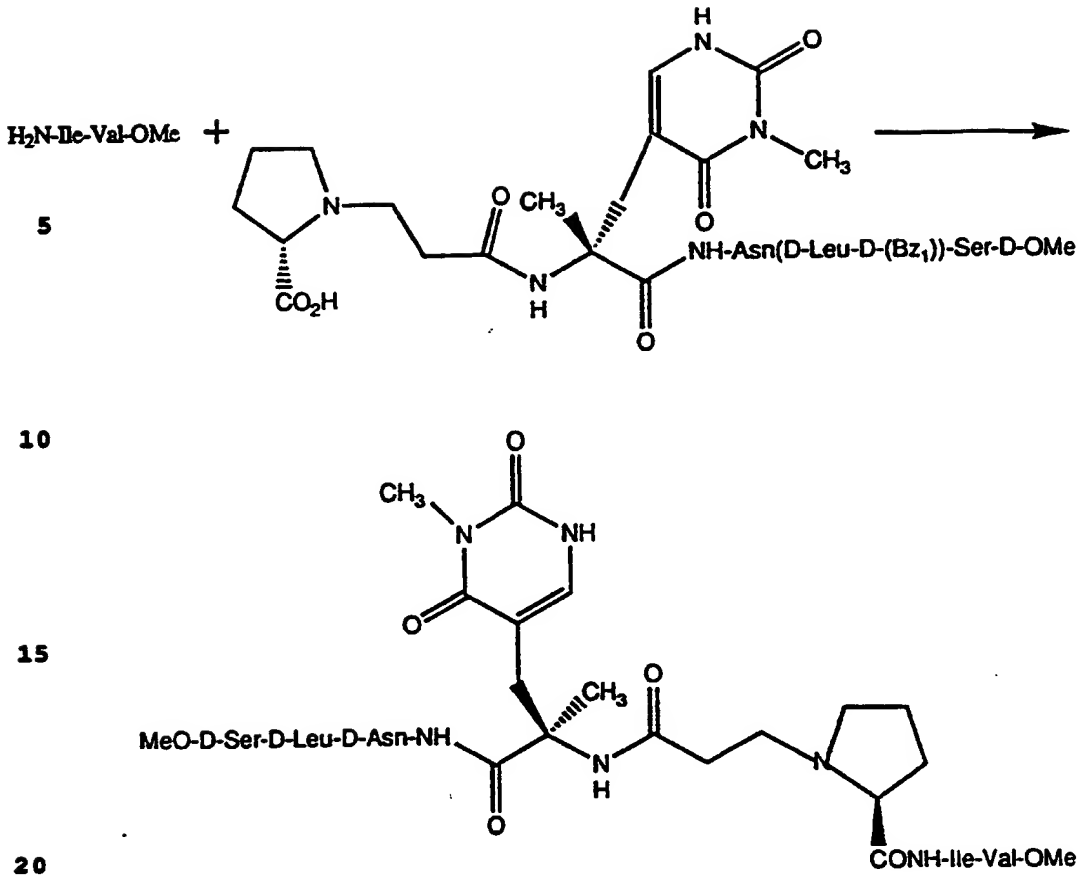
This example teaches the synthesis of a competitive inhibitor for the HIV protease, based on the insertion of a chiral azlactone residue into a strategically important position in the scissile position of the known substrate, Ac-Ser-Leu-Asn-Phe-Pro-Ile-Val-OMe. See, e.g., 33 J. Med. Chem. 1285 (1990) and references cited therein.



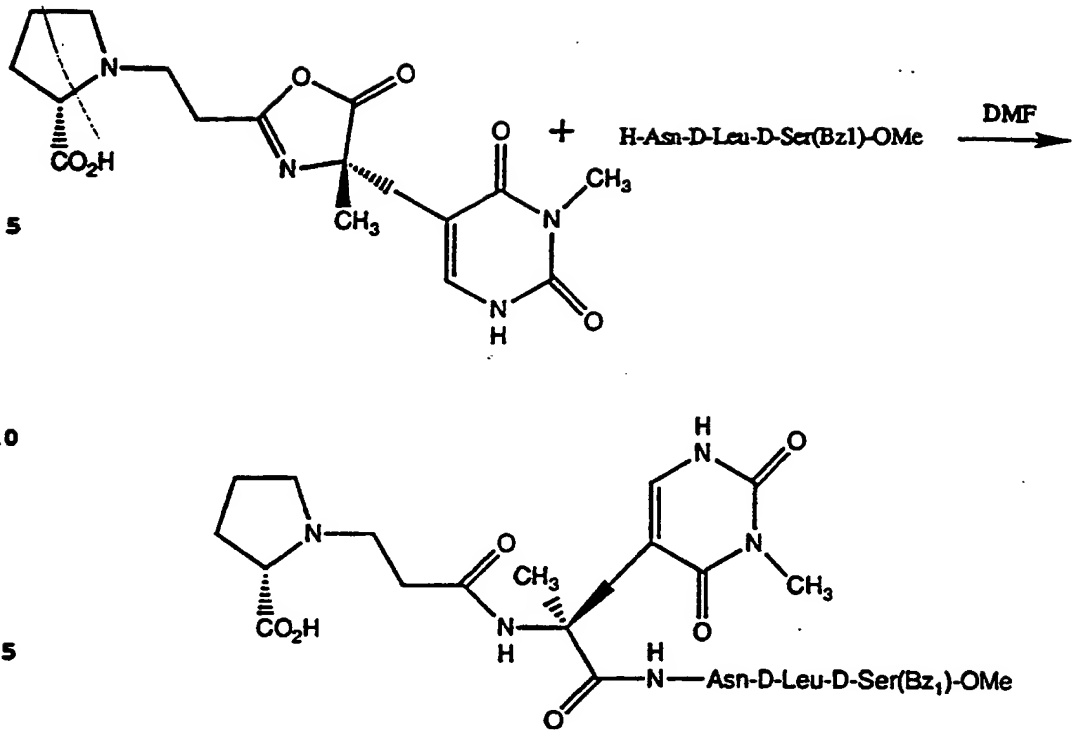
0.341 g (1 mmol) of HN-(L)-Pro-(L)-Ile-(L)-Val-OMe prepared using standard peptide-synthesis techniques, is dissolved in the minimum amount of DMF. To this mixture is added 0.229 g (1 mmol) 2-acryloyl-(S)-4-ethyl-4-benzyl-5-oxazolone described above, and the mixture is stirred at room temperature until the Michael addition reaction has proceeded to completion (as monitored by TLC). 0.393 g (1 mmol) of MeO-D-Ser(Bzl)-D-Leu-D-Asn-NH_2, prepared from the BOC-protected D-amino acids using standard peptide protection and coupling chemistries (see, e.g., J. Med. Chem. 1285 (1990) and references cited therein) is then added and the mixture is heated to 60°C and stirred at this temperature for an additional 12 hours. The DMF is then removed under high vacuum and the residue is purified by standard C18 reverse-phase chromatography to yield the protected peptide. The side-chain blocking groups are subsequently removed using standard peptide deprotection techniques to yield the product MeO-D-Ser-D-Leu-D-Asn-NH-CO-(S)-Phe-[Me]-NH-CO-CH2-CH2-L-N-Pro-L-Ile-L-Val-OMe, useful as a competitive inhibitor for the HIV protease.

13. Example: Synthesis of a Mimetic Inhibitor for the HIV Protease

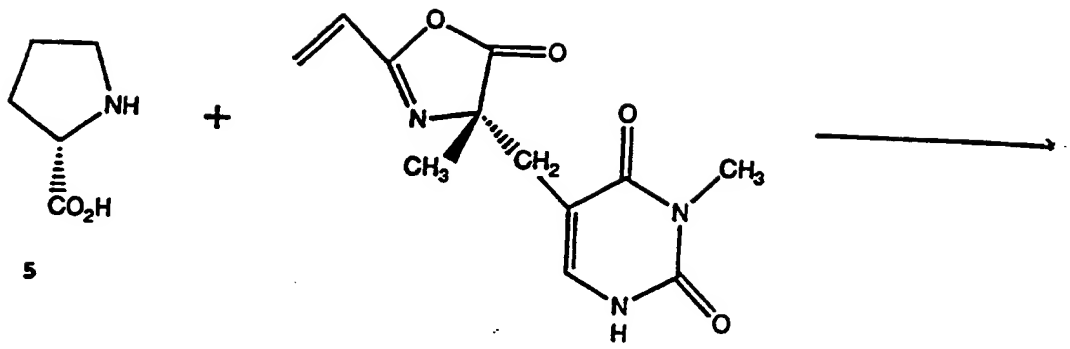
This example teaches the synthesis of another competitive inhibitor for the HIV protease. In this case the phenyl substituent is replaced with a uracil derivative.

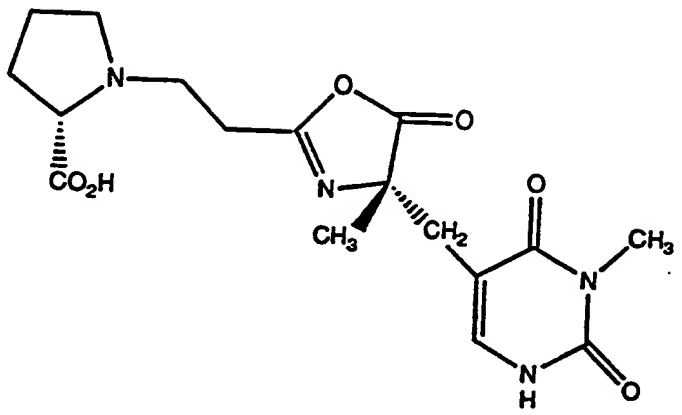


0.82 g (1 mmol) of the uracil derivative, whose preparation is described below, is coupled through the free proline carboxylic acid group to 0.244 g (1 mmol) of Ile-Val-OMe using standard peptide coupling methods. The product is purified by standard C18 reverse-phase chromatography to yield the protected peptide. The Bzl side-chain blocking group is then removed using standard deprotection techniques to yield the product shown above, useful as a competitive inhibitor for the HIV protease.

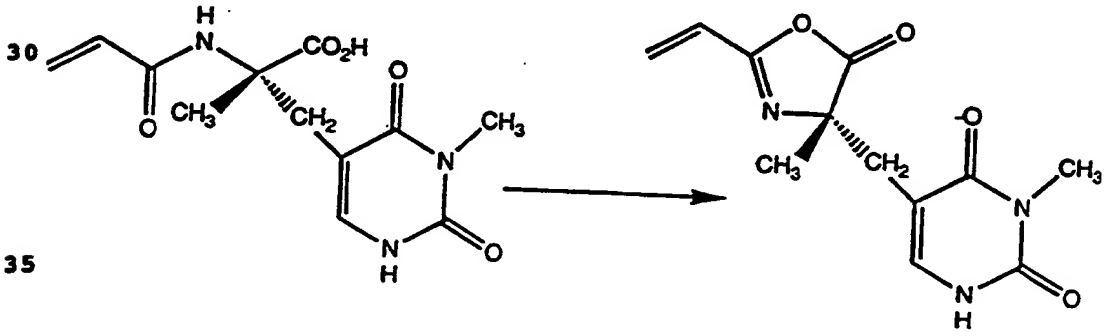


0.47 g (1 mmol) of the (S)-(S)-proline-vinylazlactone Michael adduct is dissolved in the minimum amount of DMF. 0.488 g (1 mmol) of MeO-D-Ser-(Bzl)-D-Leu-D-Asn-NH_2, prepared from the BOC-protected amino acid via standard peptide synthesis techniques (see, e.g., 33 J. Med. Chem. 1285 (1990) and references cited therein) is then added and the mixture is heated to 60°C and stirred at this temperature for 12 hours. The DMF is then removed under high vacuum to yield 0.95 g of crude product.

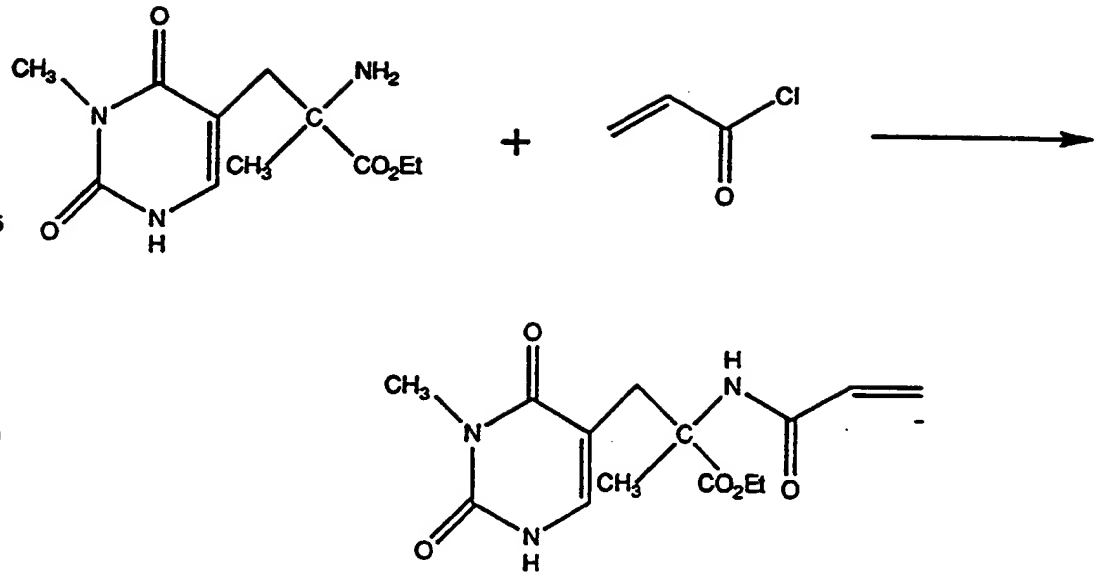




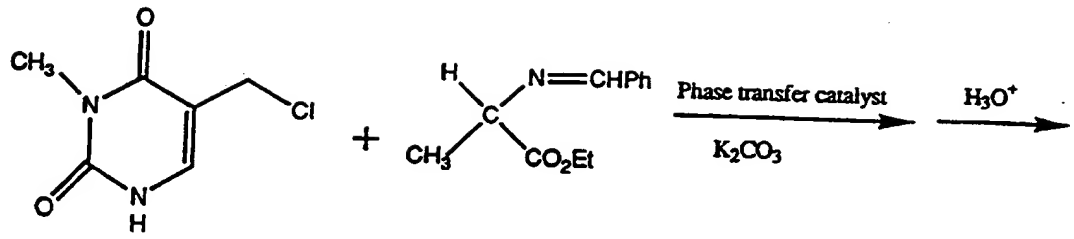
2.33 g (5 mmol) of L-proline is dissolved in the minimum amount of DMF, 1.75 g (5 mmol) of racemic uracil-functionalized azlactone is added and the mixture is stirred at room temperature until the Michael addition reaction proceeds to completion (as monitored by TLC). The DMF is then removed under high vacuum and the diastereomeric mixture is purified by standard normal-phase chromatography to give the desired (S)-(S)-Michael adduct.

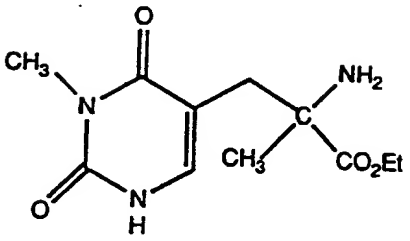


3.69 g (0.01 mol) racemic N-acryloyl-2-methyl-(3'methyluracil)-5'-alanine is stirred with 50 ml of dry acetone and 1.09 (0.01 mol) of ethyl chloroformate was added. 1.4 ml (0.01 mol) of triethylamine is added dropwise over a period of 10 min. and the mixture is stirred at room temperature until the evolution of gas ceases (1.5 hours). The triethylamine hydrochloride is removed by filtration and the cake was slurried with 20 ml of acetone and refiltered. The combined filtrates are concentrated to 50 ml on a rotary evaporator, cooled to -30°C and the crystallized product collected by filtration and dried *in vacuo* to yield racemic 4-(2-methyl-5'-[3'methyluracil])-4-methyl-2-vinylazlactone.



17.15 g (0.05 mol) of the racemic 2-(3'-methyluracil)-5'-methylalanine ethyl ester is added with stirring to a solution of 4.0 g (0.1 mol) sodium hydroxide in 100 ml water. The mixture is stirred until complete solubilization is achieved, and then cooled to 10°C. 0.05 g 2,6-di-t-butyl-p-cresol is added as a polymerization inhibitor followed by 4.52 g (0.05 mol) acryloyl chloride, which is added dropwise with stirring, keeping the temperature at 10-15°C with external cooling. To this solution is then added over a 10-min. period 5.7 ml (0.0625 mol) concentrated hydrochloric acid, again keeping the temperature at 15°C. After the addition is complete, the reaction mixture is stirred for an additional 30 min., cooled to 0°C, and the solid product is collected by filtration, washed well with ice water and pressed firmly with a rubber dam. The resulting wet cake is recrystallized from ethanol/water, and the wet cake is hydrolized with 6N HCL to yield 12.91 g (70%) of racemic N-acryloyl-(3'-methyluracil)-5'-methylalanine.





20.5 g (0.1 mol) of the Schiff base prepared from the ethyl ester of alanine and benzaldehyde according to the method of O'Donnell *et al.* (23 Terahedron Lett. 4259 (1982)) and 17.4 g (0.1 mol) of 3-methyl-5-chloromethyluracil in the mimimum amount of methylene chloride is added dropwise with stirring to a mixture of finely powdered potassium hydroxide and a catalytic amount (0.01 eq) of the phase-transfer reagent $C_6H_5CH_2NEt_3Cl$ in the same solvent at 0°C. Following addition, the mixture is stirred at 10°C until the starting material is consumed (approximately 2 hours). An aqueous workup is followed by mild acid hydrolysis of the crude with 1N HCl/Et_2O at 0°C for 3 hours to yield 29.5 g (86%) of the racemic α-methyl amino acid ester.

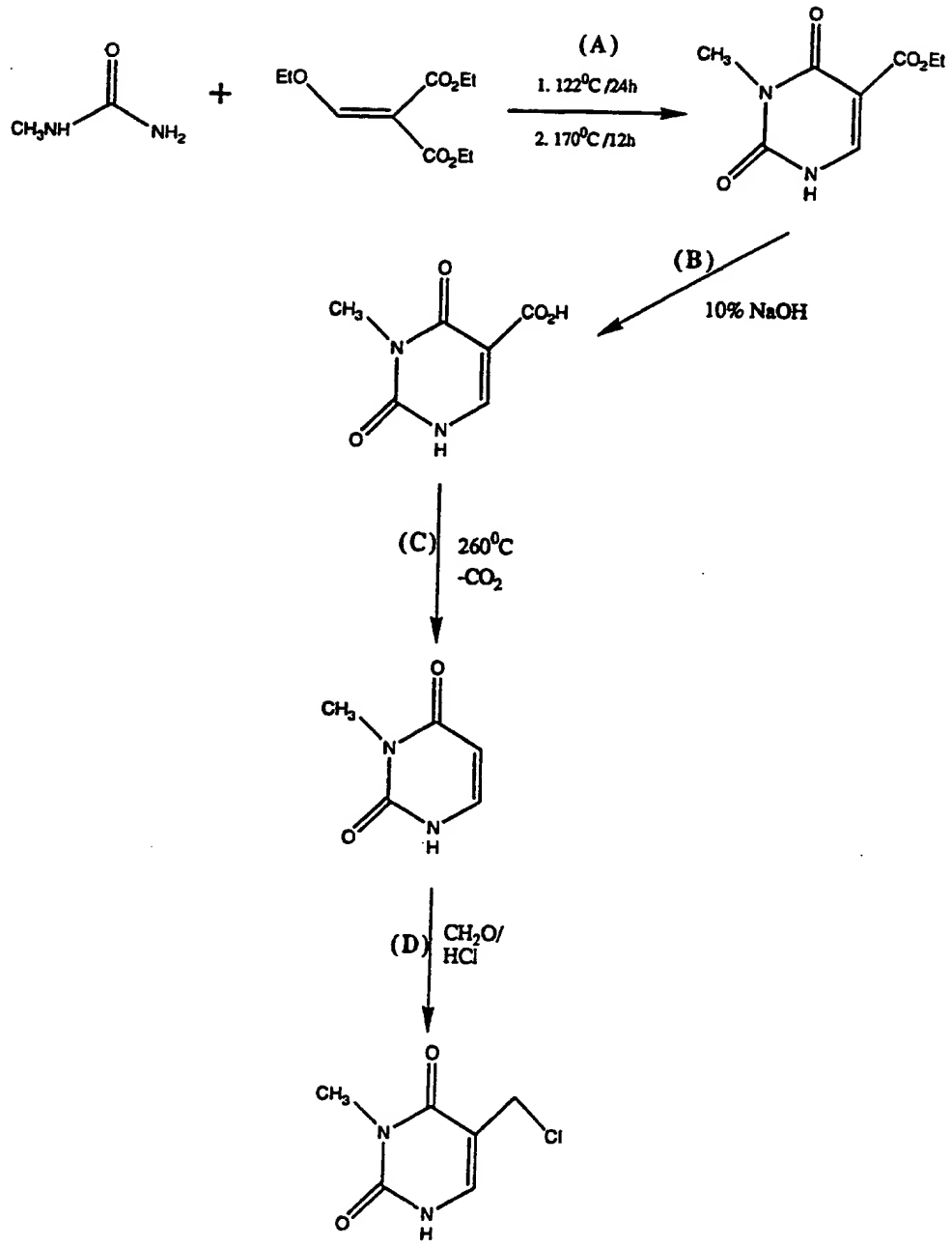
Synthesis of 3-methyl-5-chloromethyluracil

A. 74.08 g (1 mol) of N-methyl urea and 216.2 g (1 mol) of diethylethoxymethylenemalonate are heated together at 122 °C for 24 hours, followed by 170°C for 12 hours to yield the 3-methyluracil-5-carboxylic acid ethyl ester in 35% yield, following recrystallization fr m thyl acetate.

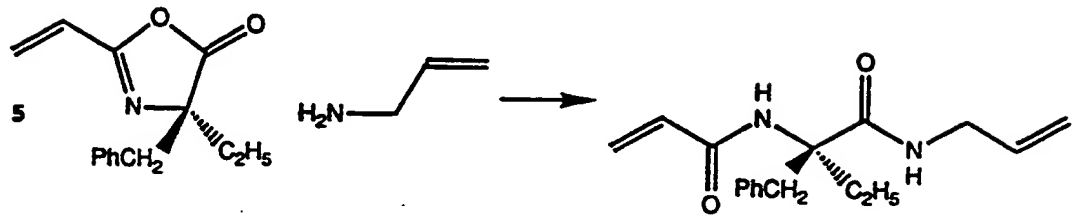
B. 30 g 3-methyluracil-5-carboxylic acid ethyl ester was saponified with 10% NaOH to give the free acid in 92% yield, after standard work-up and recrystallization from ethyl acetate.

C. 20 g of 3-methyluracil-5-carboxylic acid was decarboxylated at 260°C to give a quantitative yield of 3-methyluracil.

D. 3-methyluracil-5-carboxylic acid was treated with HCL and CH_2O using standard chloromethylation conditions to yield 3-methyl-5-chloromethyluracil in 52% yield, following standard work-up and recrystallization from ethyl acetate.


(A)
1. 122°C /24h
2. 170°C /12h
(B)
10% NaOH
(C)
260°C
-CO2
(D)
CH2O/
HCl

14. Example: Preparation of a Chiral Crosslinking Conjugate Monomer

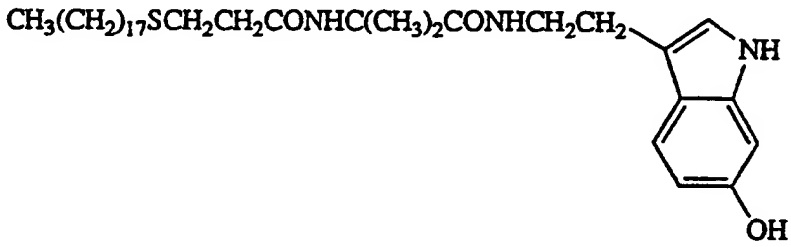


4.59 g (0.02 mol) (S)-4-ethyl,4-benzyl-2-vinyl-5-oxazolone as prepared in Example 3.3.3 above was added portionwise to a stirred solution of 1.14 g (0.02 mol) allyl amine in 75 ml of methylene chloride cooled to 0°C with an ice bath. After 15 min. the mixture was allowed to warm to room temperature, and was then stirred at room temperature for 4 hours. The solvent was stripped under aspirator vacuum on a rotary evaporator to yield 5.7 g of crude monomer, identified by NMR and FTIR analyses. The product was recrystallized from ethyl acetate to yield pure white crystalline monomer, useful for fabricating crosslinked chiral gels, beads, membranes and composites for chiral separations.

15. Examples: Synthesis of Conjugate Useful in Isolation and Purification of Serotonin-Binding Receptors

28.6 g (0.1 mol) of sieve-dried octadecane thiol and 13.9 g (0.1 mol) of 2-vinyl-4,4'-dimethylazlactone are mixed in a dry round-bottomed flask equipped with a magnetic stirrer and a drying tube filled with Drierite and cooled in an ice bath. After 1 hour the mixture is allowed to come to room temperature and is held at room temperature for four days. The product is then dissolved in 250 ml of a suitable solvent, the system cooled in an ice bath, and a chilled s lution of 17.62 g (0.1 mol) of serot nin in 250 ml of the same

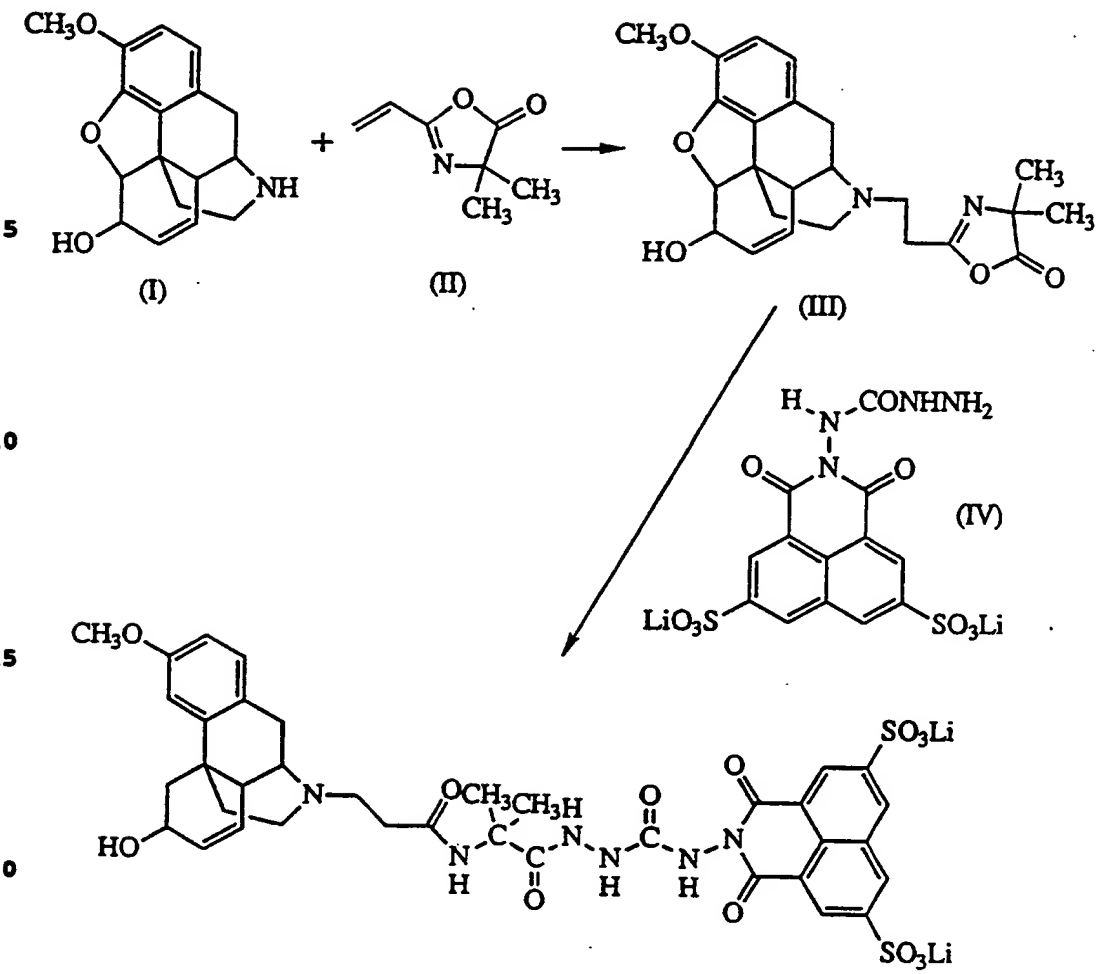
solvent is added over a 30-min period. The reaction mixture is allowed to come to room temperature over a 2-hour period and stirred at room temperature for a further 4 hours. The solvent is then removed by freeze drying to yield 60 g of the derivative



which is useful as a ligand for the stabilization and isolation of serotonin-binding membrane receptor proteins.

PRODUCT]

16. Example: Synthesis of a Conjugate Useful in the Isolation and Purification of the Morphine Receptor



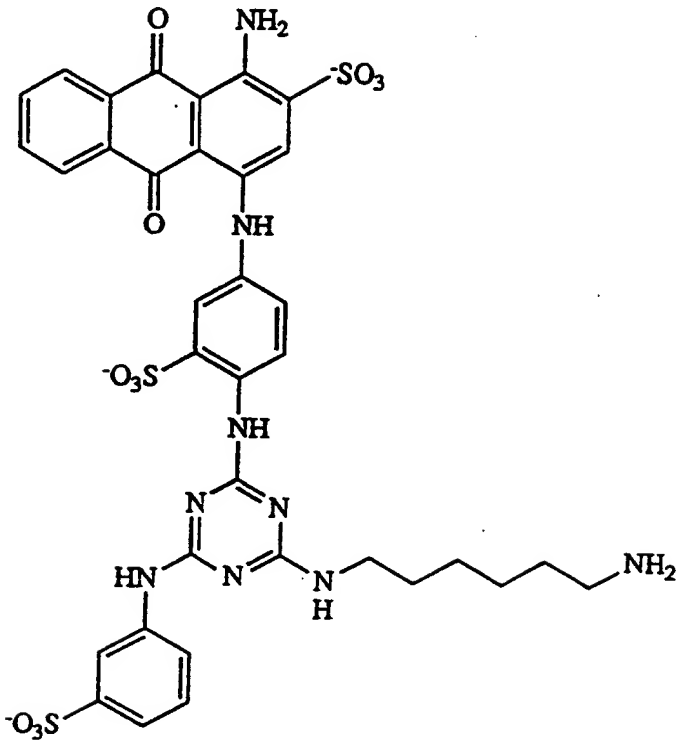
To a solution of 0.285 g (0.001 mol) of norcodeine (I) dissolved in 50 ml of the appropriate solvent, such as benzene, is added a solution of 0.139 g (0.001 mol) of 4,4'-dimethylvinylazlactone (II) in 10 ml of the same solvent. The resulting solution is heated to 70 °C and held at this temperature for 10 hours. At the end of this time the solvent is removed under vacuum to yield 0.42 g of the Michael adduct (III). 0.21 g (0.0005 mol) of this adduct is added portionwise over a 30 minute period, with stirring, to 0.23 g (0.0005 mol) of lucifer yellow-CH (IV) in 50 ml of a 1:1 mixture of water and an appropriate solvent, such as acetone, adjusted to pH 7.5.

at 0 °C under a nitrogen blanket. The reaction mixture is stirred at 0 °C for 1 hour and then allowed to come to room temperature. The mixture is then stirred at room temperature under a nitrogen blanket for 7 days. The solvent is removed under vacuum and the water is removed by freeze drying to give the product (V). (V) is useful as a probe for the study of receptor proteins that bind morphine and its derivatives.

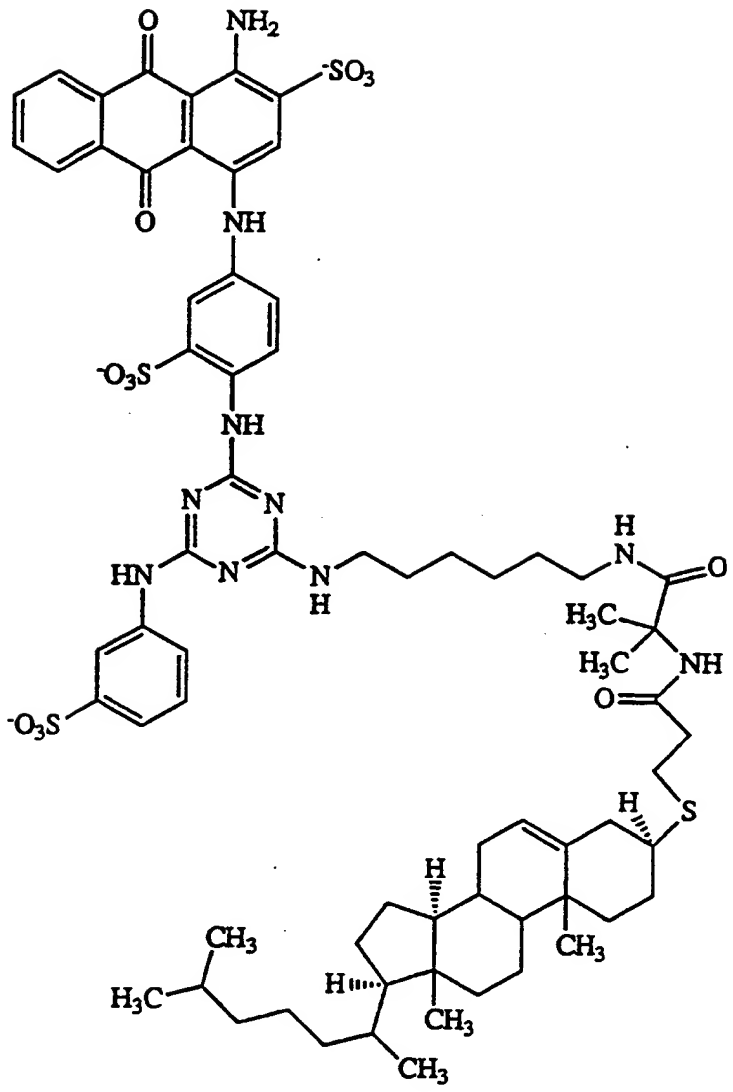
17. Example: Synthesis of Conjugate Useful in the Isolation and Purification of Proteins Binding Cibacron Blue

To 4.03 g (0.01 mol) of a stirred solution of thiocholesterol in 100 ml of an appropriate solvent, such as benzene, is added a solution of 1.39 g (0.01 mol) of 2-vinyl-4,4'-dimethyl-5-azlactone in 10 ml of the same solvent. The mixture is heated to 70 °C and stirred at this temperature for 4 hours. The solvent is completely removed under vacuum and the product (VI) is redissolved in 200 ml of dimethyl formamide. This solution is cooled in an ice bath and 8.5 g (0.01 mol) of the Cibacron Blue derivative (VII), prepared as described below, dissolved in 250 ml of DMF and 100 ml of triethylamine is added over a 30 min period. The reaction mixture is stirred with cooling for 1 hour, allowed to come to room temperature amd stirred for 12 hours. The mixture is then added to 1 liter of 25% NaCl in water at 0 °C and stirred for 15 min; then 100 ml of 10M hydrochloric acid is added with stirring and cooling, and the blue precipitate is collected by filtration, reslurried in 1 liter of water and refiltered. This extraction procedure is repeated two more times. The product (VIII) is dried at 60 °C in a vacuum oven at 30" of vacuum. (VIII) is useful for inserting and positioning the Cibacron Blue functionality, which is a broadly versatile affinity recognition ligand in cell membranes for the study of

transmembrane processes involving proteins that bind to the dye function.



VII



VIII

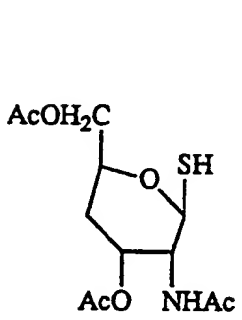
Preparation of Cibacron Blue Derivative (VIII)

40.0 g (0.05 mol) of Cibacron Blue F3 GA is dissolved in 1 liter of DMF at 40 °C with stirring. To this solution is added 26.5 g (0.23 mol) of hexamethylene diamine with stirring, followed by 4.0 g (0.05 mol) of pyridine. The reaction mixture is allowed to stir overnight and the pH is adjusted to 2.0 by the addition of 80 ml of 10M hydrochloric acid and 940 g of NaCl. 3.5 liters of water are added to precipitate the modified dye. The mixture is stirred for 1 hour and the dye is collected by filtration. The cake is washed with an additional 3.5 liters of water at pH 2.0 water and dried at 70 °C in a vacuum oven at 30" of vacuum to yield 34.0 g of the amino-functionalized dye (VII).

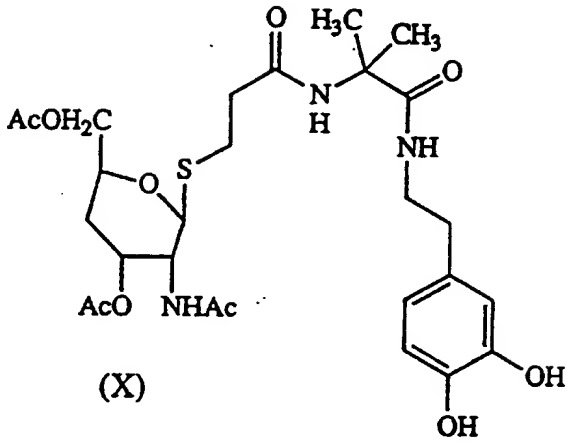
18. Example: Synthesis of a Photoreactive Conjugate Useful in the Isolation and Purification of β-N-Acetylglucosamidase

3.63 g (0.01 mol) of 2-acetamido-2-deoxy-1-thio-b-D-glucopyranose-3,4,6-triacetate (IX) and 1.39 g of 2-vinyl-4,4'-dimethylazlactone are dissolved with stirring in 100 ml of an appropriate solvent, heated to 70 °C and held at this temperature with stirring for 12 hours. At the end of this time the mixture is cooled to room temperature and 1.53 g (0.01 mol) of dopamine, dissolved in 50 ml of the same solvent is added, with cooling and stirring, over a 30 min period. The temperature is the allowed to rise to room temperature and the reaction mixture is stirred overnight. The solvent is then removed by freeze drying to produce 6.5 g of the product (X) which is useful for the study of beta-N-acetylglucosamidase and related proteins of similar specificity, since the carbohydrate functionality can bind to these proteins (See 350 Biochim. Biophys. Acta. 437 (1974)). The dopamine-connected catechol functionality is a photographic developer, capable of

photographic amplification by means of standard techniques.



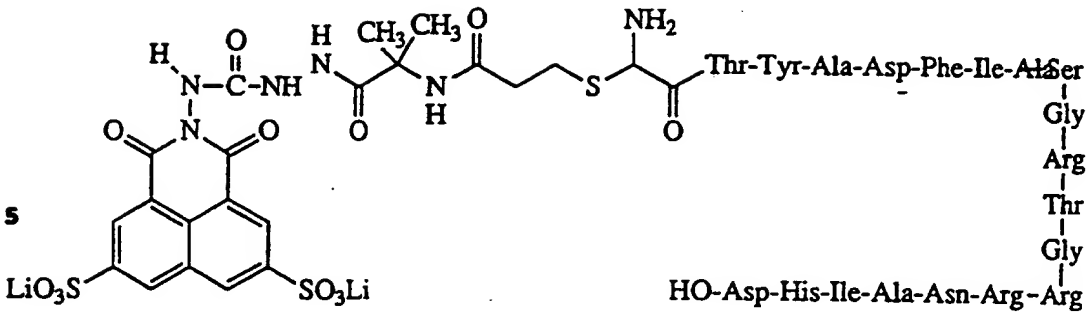
(IX)



(X)

19. Example: Synthesis of a Ligand of Protein Kinase

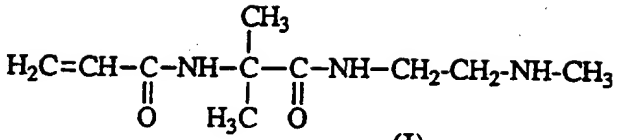
100 mg of the 20-mer cysteine variant, Cys-Thr-Tyr-Ala-Asp-Phe-Ile-Ala-Ser-Gly-Arg-Thr-Gly-Arg-Arg-Asn-Ala-Ile-His-Asp, of a protein kinase natural binding peptide ligand PK (5-24) (See, 253 Science 414 (1991)), synthesized by standard peptide synthesis techniques, is shaken with 7 mg of 2-vinyl-4,4'-dimethyl azlactone in 0.5 ml of an appropriate solvent at room temperature for 6 days. At the end of this period 23 mg of Lucifer Yellow CH in 0.5 ml of water is added, and the mixture is shaken at room temperature for 6 hours. The solvents are removed by freeze drying to yield 130 mg of the bifunctional adduct (XI), which is useful as a ligand for competitive evaluation of the binding affinity of competitive ligands for protein kinases and structurally similar proteins.



20. Example: Synthesis of Materials Useful as Coatings

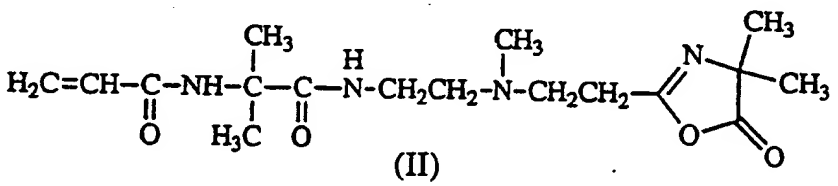
This example describes preparation of a coating by a ring-opening reaction followed by Michael-addition.

In the first synthetic step, 8.82 g (0.113 mol) of 95% N-methylethylenediamine were dissolved in 75 ml methylene chloride with stirring and cooled to 0 °C in an ice bath. Then, 13.9 g (0.10 mol) of dimethylvinylazlactone (the starting species illustrated in Eq. 3 with $R_2 = R_3 = CH_3$) pre-cooled to 0 °C were added to the methylene chloride mixture such that the temperature remained below 5 °C. The solution was then stirred at room temperature. After approximately 15 min a white precipitate began to form. The mixture was stirred for an additional 2 h at 0 °C. A white solid was collected on a Buechner funnel, washed twice with 25 ml methylene chloride and air dried to yield 13.92 g of the ring-opened adduct, identified by nuclear magnetic resonance (NMR) and Fourier transform infrared reflection (FTIR) spectroscopy as follows: NMR ($CDCl_3$): CH_3-N/gem $(CH_3)_2$ ratio 1:2; CH_2 = CH - splitting pattern in 6 ppm regioin, integration ratios and D_2O exchange experiments diagnostic for structure. FTIR (null): azlactone CO band at 1820 cm^{-1} absent; strong amide bands present in 1670 - 1700 cm^{-1} region.



(I)

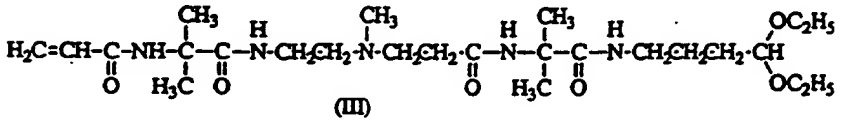
In the next synthetic step, 6.39 g (0.3 mol) of (I) and 4.17 g (0.3 mol) of dimethylvinylazlactone were dissolved in 50 ml of benzene and heated to 70 °C for 4 h. The flask was cooled to room temperature, stoppered and allowed to stand for 3 days at room temperature. The solvent was then decanted off from the thick oil that had formed. This oil was dissolved in 50 ml acetone and stripped to produce another thick oil. This latter oil was pumped on at 1 torr overnight to yield 3.53 g of a white crystalline solid, identified by NMR and FTIR spectroscopy as follows: NMR: CH_3-N/gem $(CH_3)_2$ ratio 1:4; CH_2 = CH - splitting pattern in 6 ppm region, integration ratios and D_2O exchange experiments diagnostic for structure. FTIR (null): strong azlactone CO band at 1800 cm^{-1}.



(II)

In the final synthetic step, 3.5 g (0.01 mol) of (II) and 1.61 g (0.01 mol) of $H_2N(CH_2)_3CH(OC_2H_5)_2$ were dissolved in 50 ml acetone chilled to 0 °C and stirred for 4 h at 0 °C. The solution was allowed to come to room temperature and to stand for 2 days. The resulting

yellowish solution was stripped and pumped on at 1 torr at room temperature overnight to produce 5.0 g of a white solid. 4.5 g of this solid were dissolved in hot ethyl acetate, brought to the cloud point with hot hexane and allowed to crystallize at room temperature overnight. 3.54 g of a white crystalline solid were obtained after collection by filtration and drying in a vacuum oven adjusted for a 30" vacuum at room temperature overnight. The final product was identified by NMR and FTIR spectroscopy as follows: NMR ($CDCl_3$): $CH_2 = CH -$, splitting pattern in 6 ppm region, integration ratios and D_2O exchange experiments diagnostic for structure. FTIR (mull): azlactone C0 band at 1820 cm^{-1} absent.



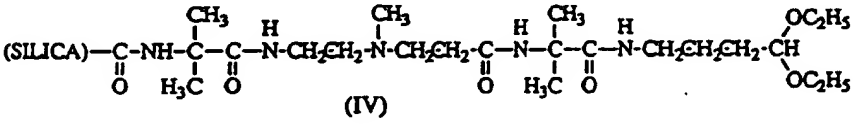
(III)

21. Example: Preparation of Coated Silica Supports Useful in Affinity Chromatography

This example describes preparation of an affinity coating from compound (III) as prepared in the previous example.

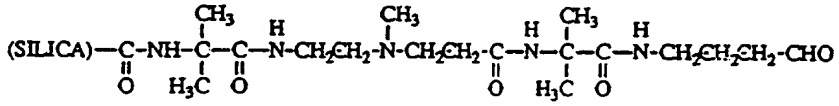
1.76 g (0.0034 mol) of (III) and 0.328 g (0.0032 mol) of n-methylol acrylamide were dissolved in 50 ml methanol, after which 1.11 ml water were added. To this solution were added 5 g of glycidoxypropyltrimethoxysilane-functionalized silica ("Epoxy Silica"). The mixture was stirred in a rotary at room temperature for 15 min and then stripped, using a bath temperature of 44 °C, to a volatiles content of 15% as measured by weight loss (from 25-200 °C with a sun gun). The silica, coated as a result of exposure to the

mixture of ingredients, was slurried in 50 ml isooctane containing 32.0 mg VAZO-64 (*i.e.*, the polymerization catalyst 2,2'-azobisisobutyronitrile dissolved in 0.5 ml toluene that had been de-aerated with nitrogen. The slurry was then thoroughly de-aerated with nitrogen and subsequently stirred at 70 °C for 2 h. The coated silica was then collected by filtration and washed three times in 50 ml methanol, and air dried. Finally, the silica was heated at 120 °C for two hours to cure the coating and yield 5.4 g of coated silica. The silica contained the following attached groups:



(IV)

1.5 g of the coated silica beads were shaken with 20 ml aqueous HCl (pH = 3.0) for 4 h at room temperature. The course of the reaction was followed by testing for the generation of free aldehyde with ammoniacal silver nitrate (Tollens test). The resulting solid was collected on a Buechner filter, then reslurried and recollected until the wash water was neutral. The silica particles were then air dried to yield 1.25 g of aldehyde packing, the terminal methoxy groups having been replaced with a single aldehyde group as follows:



Repligen Protein A was coupled to the aldehyde packing using the standard conditions given for the attachment of Bovine Serum Albumin in the accompanying instructions (Technical Note No. 4151) from Chromatochem Inc., Missoula, MT.

A one-cm glass column was packed with the Protein-A functionalized material and loaded with human IgG from PBS buffer (pH = 7.4) at a flow rate of 1.6 ml/min. The IgG was eluted in 0.01M NaOAc (pH = 3.0). The IgG was then collected and the amount measured spectrophotometrically using standard calibration curves. The measured capacity of the packing was 12 mg IgG per ml of column volume.

22. Example: Functionalization of Azlactone-Containing Polymers

It is possible to procure existing azlactone-functionalized polymeric surfaces (e.g., as described in U.S. Patent No. 4,737,560) and to functionalize them according to the steps outlined above. For example, by using successive reactions with dinucleophilic species of the form HNu^1-Z-Nu^2H and suitable azlactones, a surface of the form

(SURFACE)-(X)-Az,

where X is a linker and Az stands for axlactone, can be transformed into the species

(SURFACE)-(X)-CONHC(CH_3)$_2$CONu^1(Z)Nu^2CH_2CH_2-Az

which may be linked, if desired, to a biomolecule to form the following conjugate:

(SURFACE)
\
(X)-CONHC(CH_3)$_2$CONu^1(Z)Nu^2CH_2CH_2CONHC(CH_3)$_2$CO-Biomolecule

A suitable experimental procedure is as follows. The azlactone-functional support is slurried in a suitable solvent, such as $CHCl_3$, and cooled to 0 °C. An amount of the bifunctional nucleophile equivalent on a molar basis to the total number of surface azlactone groups present, is dissolved in the same solvent and added with shaking. The mixture is then shaken at 0 °C for 6 hours, allowed to come to room temperature, and shaken at room temperature overnight. The support is collected by filtration, washed with fresh solvent, re-slurried in an appropriate solvent and one equivalent of vinylazlactone, dissolved in the same solvent, is added thereto. The mixture is then shaken, heated to 70 °C and held at this temperature for 12 hours. At the end of this time, the mixture is cooled and the support collected by filtration. The support is then washed thoroughly with fresh solvent and dried *in vacuo*.

23. Example: Preparation of a Support Useful in the Purification of Human IgG from Serum

The functional beads prepared as above are suspended in pH 7.5 aqueous phosphate buffer. A solution of protein A (Repligen) in 10 mM phosphate buffer (pH 7.0) and at a concentration of 10 mg/900 µl is added, and the mixture is then gently shaken at room temperature for 3 hours. The beads are concentrated by centrifugation, the supernate decanted off and the beads washed five times with pH 7.5 aqueous phosphate buffer. The beads are then loaded into a 0.46 cm inner-diameter glass

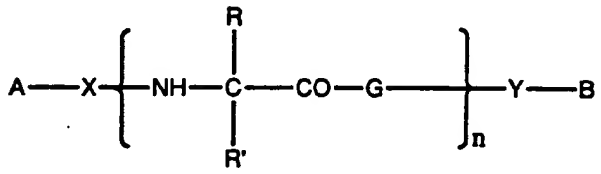
column and used to purify human IgG fr m serum using standard affinity-purification techniques.

It should be apparent to those skilled in the art that other compositions and processes for preparing the compositions not specifically disclosed in the instant specification are, nevertheless, contemplated thereby. Such other compositions and processes are considered to be within the scope and spirit of the present invention. hence, the invention should not be limited by the description of the specific embodiments disclosed herein but only by the following claims.

THE CLAIMS

What is claimed is:

1. A composition having the structure:



wherein:

a. A and B are the same or different, and each is a chemical bond; hydrogen; an electrophilic group; a nucleophilic group; R; an amino acid derivative; a nucleotide derivative; a carbohydrate derivative; an organic structural motif; a reporter element; an organic moiety containing a polymerizable group; or a macromolecular component, wherein A and B are optionally connected to each other or to other structures and R is as defined below;

b. X and Y are the same or different and each represents a chemical bond or one or more atoms of carbon, nitrogen, sulfur, oxygen or combinations thereof;

c. R and R' are the same or different and each is an alkyl, cycloalkyl, aryl, aralkyl or alkaryl group or a substituted or heterocyclic derivative thereof, wherein R and R' may be different in adjacent n units and have a selected stereochemical arrangement about the carbon atom to which they are attached;

d. G is a connecting group or a chemical bond which may be different in adjacent n units; and

e. $n \geq 1$;

provided that, (1) if n is 1, and X and Y are chemical bonds, A and B are different and one is other than a chemical bond, H or R, and A and B each is other than an amino acid residue or a peptide; (2) if n is 1 and Y is a chemical bond, G includes a NH, OH or SH terminal group for connection to the carbonyl group and G-B is not an amino acid residue or a peptide; (3) if n is 1 and X, Y, and G each is a chemical bond, A and B each is other than a chemical bond, an amino acid residue or a peptide; and (4) if n is 1, either X or A has to include a CO group for direct connection to the NH group.

2. The composition of claim 1 wherein G is chemical bond or the ring-opening reaction product of a nucleophilic group and an oxazolone and n > 2.

3. The composition of claim 1 wherein at least one of R and R' includes a hydroxyl containing substituent.

4. The composition of claim 1 wherein X is a carbonyl group.

5. The composition of claim 1 wherein G includes a NH, OH or SH terminal group for connection to the carbonyl group.

6. The composition of claim 1 wherein G is a chemical bond and Y is a compound which includes a NH, OH or SH terminal group.

7. The composition of claim 1 wherein G is a chemical bond, Y is an oxygen atom and B is a hydrogen.

8. The composition of claim 1 wherein G includes at least one of an aromatic ring, a heterocyclic ring, a carbocyclic moiety, an alkyl group or a substituted derivative thereof.

9. The composition of claim 1 wherein A and B are th same.

10. The c mposition of claim 1 where R and R' are different so that the composition is chiral.

11. The compositi n of claim 1 wherein at least one of A and B is a terminal-structural moiety of formula T-U, wherein:

a. U is selected from the group consisting of aliphatic chains having from 2 to 6 carbon atoms, substituted or unsubstituted aryl, substituted or unsubstituted cycloalkyl, and substituted or unsubstituted heterocyclic rings; and

b. T is selected from the group consisting of OH, NH_2, SH, $(CH_3)_3N^+$, $-SO^-_3$, COO^-, CH_3, H, and phenyl.

12. The composition of claim 11 wherein at least one of A and B is $HO-CH_2-(CHOH)_n$ where n is an integer.

13. The composition of claim 1 wherein A and B are part of the same cyclic moiety.

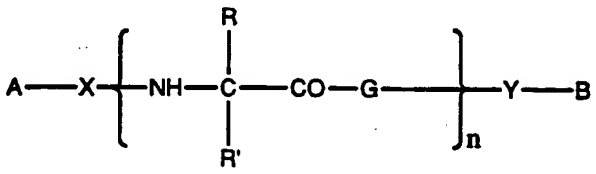
14. The composition of claim 1 wherein n is 1 and G includes a NH, OH or SH terminal group for connection to the carbonyl group.

15. The composition of matter of claim 14 wherein G is a group containing the atom of the nucleophile used in the ring-opening reaction of an oxazolone.

16. The composition of claim 14 wherein R and R' are different so that the composition is chiral.

17. The composition of claim 1 wherein R and R' are different, X is a chemical bond and A is nucleotide derivative; a carbohydrate derivative; an organic structural motif; a reporter element; an organic moiety containing a polymerizable group; or a macromolecular component.

18. A peptide mimetic having the structure



wherein:

a. A and B are the same or different, and at least one is an amino acid derivative of the form $(AA)_m$, wherein AA is a natural or synthetic amino acid residue and m is an integer, and A and B are optionally connected to each other or to other structures;

b. X and Y are the same or different and each represents a chemical bond or one or more atoms of carbon, nitrogen, sulfur, oxygen or combinations thereof;

c. R and R' are the same or different and each is an alkyl, cycloalkyl, aryl, aralkyl or alkaryl group or a substituted or heterocyclic derivative thereof, wherein R and R' may be different in adjacent n units and have a selected stereochemical arrangement about the carbon atom to which they are attached;

d. G is a connecting group or a chemical bond which may be different in adjacent n units; and

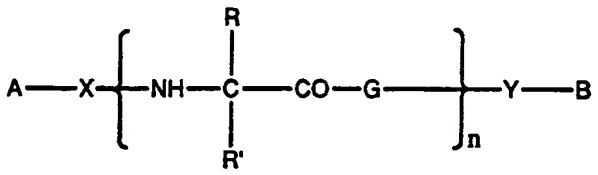
e. $n \geq 1$;

provided that, when (1) n is 1 and Y is a chemical bond, G includes a NH, OH or SH terminal group for connection to the carbonyl group and G-B is not an amino acid residue or a peptide; (2) if n is 1 and X, Y, and G each is a chemical bond, A and B each is other than a chemical bond, an amino acid residue or a peptide; and (3) if n is 1, either X or A has to include a CO group for direct connection to the NH group.

19. The composition of claim 1 wherein G is (1) Nu^1-Y-P where Nu^1 is a nucleophilic group, Y is as defined above and P is a reactive group optionally containing a protective group; or
(2) as α-α-di-substituted amino acid residue.

20. The composition of claim 19 wherein P is a nucleophilic group optionally containing a protective group.

21. A nucleotide mimetic having the structure:



wherein:

a. A and B are the same or different, and at least one is a nucleotide derivative, wherein A and B are optionally connected to each other or to other structures;

b. X and Y are the same or different and each represents a chemical bond or one or more atoms of carbon, nitrogen, sulfur, oxygen or combinations thereof;

c. R and R' are the same or different and each is an alkyl, cycloalkyl, aryl, aralkyl or alkaryl group or a substituted or heterocyclic derivative thereof, wherein R and R' may be different in adjacent n units and have a selected stereochemical arrangement about the carbon atom to which they are attached;

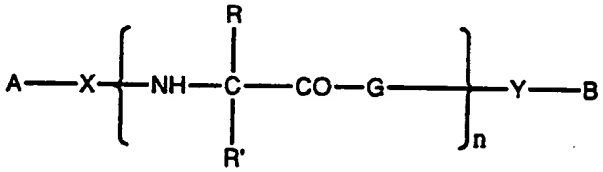
d. G is a connecting group or a chemical bond which may be different in adjacent n units; and

e. $n \geq 1$;

provided that, when n is 1 and Y is a chemical bond, G includes a NH, OH or SH terminal group for connection to the carbonyl group.

22. The nucleotide mimetic of claim 21 wherein A is a nucleotide derivate of the form $(NUCL)_l$, wherein l is an integer, such that $(NUCL)_l$ is a natural or synthetic nucleotides when l=1, a nucleotide probes when l=2-25 and an oligonucleotides when l>25 including both deoxyribose (DNA) and ribose (RNA) variants.

23. A carbohydrate mimetic having the structure:



wherein:

a. A and B are the same or different, and at least one is a carbohydrate derivative; wherein A and B are optionally connected to each other or to other structures;

b. X and Y are the same or different and each represents a chemical bond or one or more atoms of carbon, nitrogen, sulfur, oxygen or combinations thereof;

c. R and R' are the same or different and each is an alkyl, cycloalkyl, aryl, aralkyl or alkaryl group or a substituted or heterocyclic derivative thereof, wherein R and R' may be different in adjacent n units and have a selected stereochemical arrangement about the carbon atom to which they are attached;

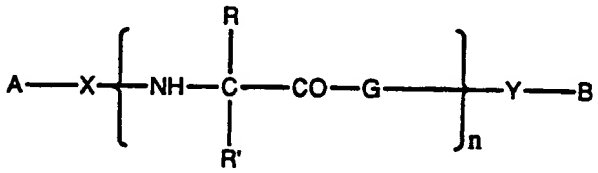
d. G is a connecting group or a chemical bond which may be different in adjacent n units; and

e. $n \geq 1$;

provided that, when n is 1 and Y is a chemical bond, G includes a NH, OH or SH terminal group for connection to the carbonyl group.

24. The carbohydrate mimetic of claim 23 wherein A and B each is a natural carbohydrate, a synthetic carbohydrate residue or derivative thereof or a related organic acid thereof.

25. A pharmaceutical compound having the structure:



wherein:

a. A and B are the same or different, and at least one is an organic structural motif; wherein A and B are optionally connected to each other or to other structures;

b. X and Y are the same or different and each represents a chemical bond or one or more atoms of carbon, nitrogen, sulfur, oxygen or combinations thereof;

c. R and R' are the same or different and each is an alkyl, cycloalkyl, aryl, aralkyl or alkaryl group or a substituted or heterocyclic derivative thereof, wherein R and R' may be different in adjacent n units and have a selected stereochemical arrangement about the carbon atom to which they are attached;

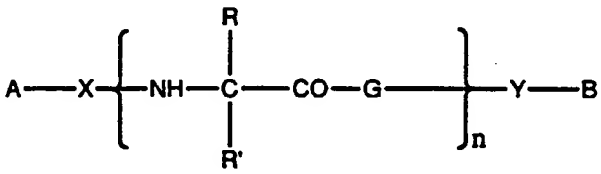
d. G is a connecting group or a chemical bond which may be different in adjacent n units; and

e. $n \geq 1$;

provided that, when n is 1 and Y is a chemical b nd, G includes a NH, OH or SH terminal group for connection to the carbonyl group.

26. The pharmaceutical compound of claim 25 wherein the structural motif of the organic compound is obtained from a pharmaceutical compound or a pharmacophore or metabolite thereof and has specific binding properties to ligands.

27. A reporter compound having the structure:



wherein:

a. A and B are the same or different, and at least one is a reporter element; wherein A and B are optionally connected to each other or to other structures;

b. X and Y are the same or different and each represents a chemical bond or one or more atoms of carbon, nitrogen, sulfur, oxygen or combinations thereof;

c. R and R' are the same or different and each is an alkyl, cycloalkyl, aryl, aralkyl or alkaryl group or a substituted or heterocyclic derivative thereof, wherein R and R' may be different in adjacent n units and have a selected stereochemical arrangement about the carbon atom to which they are attached;

d. G is a connecting group or a chemical bond which may be different in adjacent n units; and

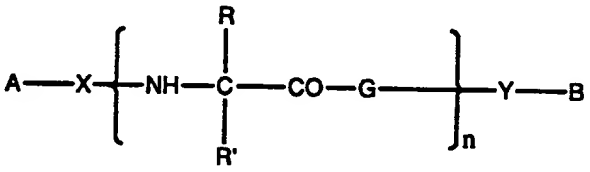
e. $n \geq 1$;

provided that, when n is 1 and Y is a chemical bond, G includes a NH, OH or SH terminal group for connection to the carbonyl group.

28. The reporter compound of claim 27 wherein the reporter element is a natural or synthetic dye or a photographically active residues which possesses reactive groups which may be synthetically incorporated into the oxazolone structure or reaction scheme and may be attached through the groups without adversely interfering with the reporting functionality of the group.

29. The reporter compound of claim 28 wherein the reactive group is amino, thio, hydroxy, carboxylic acid, acid chloride, isocyanate alkyl halide, aryl halide or an oxirane group.

30. A polymerizable compound having the structure:



wherein:

a. A and B are the same or different, and at least one is an organic moiety containing a polymerizable group; wherein A and B are optionally connected to each other or to other structures;

b. X and Y are the same or different and each represents a chemical bond or one or more atoms of carbon, nitrogen, sulfur, oxygen or combinations thereof;

c. R and R' are the same or different and each is an alkyl, cycloalkyl, aryl, aralkyl or alkaryl group or a substituted or

heterocyclic derivative thereof, wherein R and R' may be different in adjacent n units and have a selected stereochemical arrangement about the carbon atom to which they are attached;

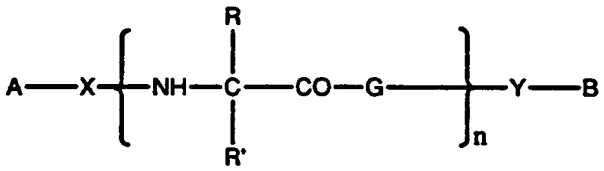
d. G is a connecting group or a chemical bond which may be different in adjacent n units; and

e. $n \geq 1$;

provided that, when n is 1 and Y is a chemical bond, G includes a NH, OH or SH terminal group for connection to the carbonyl group.

31. The polymerizable compound of claim 30 wherein the polymerizable group of the organic moiety is a vinyl group, oxirane group, carboxylic acid, acid chloride, ester, amide, lactone or lactam.

32. A substrate having the structure:



wherein:

a. A and B are the same or different, and at least one is a macromolecular component, wherein A and B are optionally connected to each other or to other structures;

b. X and Y are the same or different and each represents a chemical bond or one or more atoms of carbon, nitrogen, sulfur, oxygen or combinations thereof;

c. R and R' are the same or different and each is an alkyl, cycloalkyl, aryl, aralkyl r alkaryl group or a substituted or

heterocyclic derivative there f, wherein R and R' may be different in adjacent n units and have a selected stereochemical arrangement about the carbon atom to which they are attached;

d. G is a connecting group or a chemical bond which may be different in adjacent n units; and

e. $n \geq 1$;

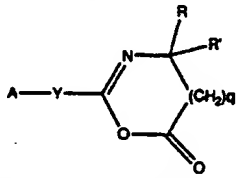
provided that, when n is 1 and Y is a chemical bond, G includes a NH, OH or SH terminal group for connection to the carbonyl group.

33. The substrate of claim 32 wherein the macromolecular component is a surface or structures which is attached to the oxazolone module via a reactive group in a manner where the binding of the attached species to a ligand-receptor molecule is not adversely affected and the interactive activity of the attached functionality is determined or limited by the macromolecule.

34. The substrate of claim 32 wherein the macromolecule component has a molecular weight of at least about 1000 Daltons.

35. The substrate of claim 32 wherein the molecular component is in the form of an ceramic particle, a nanoparticle, a latex particle, a porous or non-porous beads, a membrane, a gel, a macroscopic surface or a functionalized or coated version or composite thereof.

36. A composition having the structure:



wherein:

a. A is a chemical bond; hydrogen; an electrophilic group; a nucleophilic group; R; an amino acid derivative; a nucleotide derivative; a carb hydrate derivative; an

organic structural motif; a report r element; an organic moiety containing a polymerizable group; or a macromolecular component, wherein R is as defined below;

b. Y is a chemical bond or one or more atoms of carbon, nitrogen, sulfur, oxygen or combinations thereof;

c. R and R' are the same or different and each is an alkyl, cycloalkyl, aryl, aralkyl or alkaryl group or a substituted or heterocyclic derivative thereof, wherein R and R' may be different in adjacent n units and have a selected stereochemical arrangement about the carbon atom to which they are attached; and

d. q = 0 or 1.

37. The composition of claim 36 wherein Y includes at least one nucleophilic species which includes a nitrogen, oxygen or sulfur group attached to a $-(CH_2)_n-$ group where n is 1-2, and R and R' are the same or different and each is hydrogen, or an alkyl, cycloalkyl, aryl, aralkyl or alkaryl group, or a carbocyclic or heterocyclic ring.

38. The composition of claim 36 where in Y is a chemical bond and q = 0 or Y is;

$$(RING) - (CH_2)_n$$

where n=0-4 and (RING) designates a disubstituted phenyl ring or a substituted or unsubstituted aromatic, heterocyclic or alicyclic ring having 6-20 carbons, wherein A is a protecting group when Y contains a terminus which can react with the oxazolone ring.

39. A method of synthesizing a compound of the formula:

$B-Y-(CO-CRR'-NH)_n-H$

wherein

a. B is an amino acid derivative; a nucleotide derivative; a carbohydrate derivative; an organic structural motif; a reporter element; an organic moiety containing a polymerizable group; or a macromolecular component;

b. Y represents a chemical bond or one or more atoms of carbon, nitrogen, sulfur, oxygen or combinations thereof;

c. R and R' are the same or different and each is an alkyl, cycloalkyl, aryl, aralkyl or alkaryl group or a substituted or heterocyclic derivative thereof, wherein R and R' may be different in adjacent n units; and

e. $n \geq 2$;

which method comprises the steps of:

providing a first amino-blocked oxazolone of the formula:

$B_1-NH-CRR'-$oxazolone ring with R and R'

reacting the first amino-blocked oxazolone under conditions that promote ring-opening with a compound that includes B and has a ring opening reactive moiety to form an amino-blocked ring-opened adduct; and

deblocking the adduct by removing the amino-blocking group.

40. The method of claim 39 which further comprises:

providing a free amino group on the deblocked adduct;

providing a second amino-blocked oxazolone;

reacting the free amino group of the adduct with the second amino-blocked oxazolone to form a second adduct; and

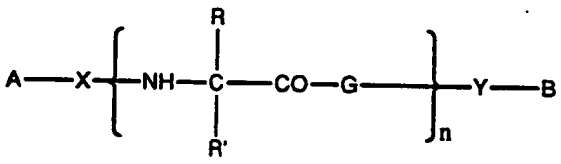
repeating the preceding steps, if necessary, to provide the desired structure of the composition.

41. The method of claim 39 which further comprises selecting the compound that is to react with the first oxazolone to include an amine, hydroxyl or sulfhydryl group to promote the ring opening; and selecting R and R' to be different so that a chiral molecule is obtained.

42. The method of claim 39 wherein the starting materials used are achiral or not enantiomerically pure.

43. The method of claim 39 further comprising the step of reacting the free amino group of the oxazolone with a carboxyl terminus of a peptide.

44. A method of synthesizing a compound of the form:



wherein

a. A and B are the same r different, and each is an amino acid derivative; a nucleotide derivative; a carbohydrate derivative; an organic structural motif; a reporter element; an organic moiety containing a polymerizable group; or a macromolecular component, wherein A and B are optionally connected to each other or to other structures;

b. X and Y are the same or different and each represents a chemical bond or one or more atoms of carbon, nitrogen, sulfur, oxygen or combinations thereof;

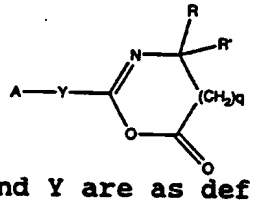
c. R and R' are the same or different and each is an alkyl, cycloalkyl, aryl, aralkyl or alkaryl group or a substituted or heterocyclic derivative thereof, wherein R and R' may be different in adjacent n units and have a selected stereochemical arrangement about the carbon atom to which they are attached;

d. G is a connecting group or a chemical bond which may be different in adjacent n units; and

e. $n \geq 1$;

wherein the method comprises the steps of:

providing an oxazolone of the formula:



where A, R, R' and Y are as defined above and q=0 or 1; and

reacting the oxazolone under conditions that promote ring-opening with a compound that includes B and has a ring opening reactive moiety to form a ring-opened adduct.

45. The method of claim 44 which further c mprises:

carrying out an appropriate subsequent reaction on the previous ring-opened product, wherein the subsequent reaction is:

1) in the case where G is a chemical bond, cyclizing the terminal α,α-asymmetrically disubstituted amino acid to form a terminal azlactone ring;

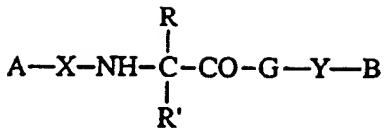
2) in the case where G is -Nu-Z where Nu is a group which includes sulfur, nitrogen or oxygen and Z includes a carboxyl carboxyl, isocyanate or acid halide terminus, adding the terminus of Z to the amino terminus of an α,α'-asymmetrically disubstituted amino acid and then cyclizing the resulting amino acid to form a terminal oxazolone ring; or

3) in the case where G is $-Nu_1-Z-Nu_2-CH_2CH_2-CO-$ where Nu^1 and Nu^2 each is a group which includes sulfur, nitrogen or oxygen and Z is a connecting group, reacting the Nu_2 terminus with the vinyl group of a 4,4'-asymmetrically disubstituted 2-vinyl oxazolone under conditions that promote a Michael addition reaction to form a terminal oxazolone ring;

repeating the preceding steps, if necessary, to provide the desired structure of the composition; and

reacting the terminal oxazolone ring with a species of the form G^o-B-YH to form the composition.

46. A method of synthesizing a compound of the formula:



wherein

a. A and B are the same or different, and each is an amino acid derivative; a nucleotide derivative; a carbohydrate derivative; an organic structural motif; a reporter element; an organic moiety containing a polymerizable group; or a macromolecular component, wherein A and B are optionally connected to each other or to other structures;

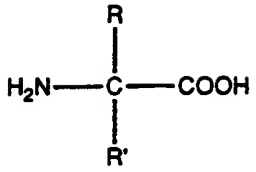
b. X and Y are the same or different and each represents a chemical bond or one or more atoms of carbon, nitrogen, sulfur, oxygen or combinations thereof;

c. R and R' are the same or different and each is an alkyl, cycloalkyl, aryl, aralkyl or alkaryl group or a substituted or heterocyclic derivative thereof, wherein R and R' have a selected stereochemical arrangement about the carbon atom to which they are attached; and

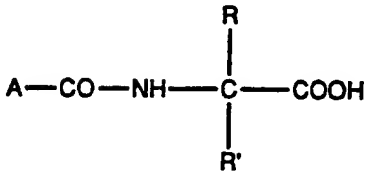
d. G is a connecting group or a chemical bond;

wherein the method comprises the steps of:

r acting an amino acid f the form



wherein R and R' are as recited above, with a carboxylic acid, an acid halide or an oxazolone to form an adduct of the formula:



cyclizing the adduct to form an oxazolone;

reacting the oxazolone with a bifunctional species of the form HX-Z-Y, wherein HX includes an amine, hydroxyl or sulfhydryl group and Y contains a reactive group capable of bonding with species B; and

reacting the resultant product with species B.

47. The method of claim 46 wherein the peptide sequence is chiral.

48. A method of synthesizing a compound containing a pepide sequence which comprises the steps of:

providing a substrate bound, via a CO group, to the amino terminus of an α,α'-disubstituted chiral amino acid;

cyclizing the amino acid into an oxazolone;

reacting the oxazolone with an alkali-metal salt of a second α,α'-disubstituted chiral amino acid to form a bound dipeptide salt;

cyclizing the second α,α'-disubstituted chiral amino acid;

repeating steps (c) and (d), if neceassary, to f rm the desired peptide sequence.

49. The method of claim 48 wherein the structure of the composition contains achiral centers or the composition is not obtained chirally pure.

50. The method of claim 49 which further comprises the step of releasing the composition from the substrate.

51. The method of claim 48 which further comprises the step of reacting a cyclized oxazolone intermediate with a species containing a reactive moiety of an amine, hydroxyl or sulfhydryl group.

52. The method of claim 48 wherein Y-Z-B is an aminimide.

53. The method of claim 48 wherein the peptide sequence is chiral.

54. A compound produced by the method of any one of claims 39 to 53.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/06240

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C08G 69/10; C07D 263/00; C07C 229/00
US CL :528/328; 548/215; 562/433, 575

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 528/328; 548/215; 562/433, 575

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 5,039,813 (FAZIO ET AL.) 13 AUGUST 1991. See column 4, lines 1-64; column 13, line 29 to column 14, line 36.	1, 2, 5, 6, 8-10, 14-16, 19, 20, 36-38
P,X	US, A, 5,219,731 (SIH) 15 JUNE 1993. See column 4, line 54 to column 5, line 33; column 6, lines 1-8.	1, 4-6, 9, 10, 14-16, 19, 20
X	US, A, 4,612,388 (MITA ET AL.) 16 SEPTEMBER 1986. See column 2, lines 30-50.	1, 3-10, 14-16, 19, 20
X	US, A, 4,125,519 (GOODMAN ET AL.) 14 NOVEMBER 1978. See column 2, lines 36-56.	1-6, 8-10, 19, 20
X	US, A, 4,996,292 (FOX ET AL.) 26 FEBRUARY 1991. See the Abstract.	1-6, 8-10, 19, 20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 24 SEPTEMBER 1993	Date of mailing of the international search report NOV 12 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer JEFFREY MULLIS Telephone No. (703) 308-2351

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/06240

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.: 48-54
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claim 48 recites that steps "(c)" and "(d)" should be repeated but does not recite what steps "(c)" and "(d)" are.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-6, 8-10, 13-16, 19, 20, 36-38

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/06240

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

1. This International Search Authority has found 121 inventions claimed in the International Application covered by the claims indicated below:

The generic claims contain the following patentably distinct species of units A and B.

Species of A and B

1) a chemical bond

2) hydrogen

3) an electrophilic group

4) R

5) an amino acid derivative

6) a carbohydrate derivative

7) an organic structural motif

8) a reporter element

9) an organic moiety containing polymerizable group
10) a macromolecular component 11) a nucleophilic group

The various combinations of A and B makes a total of 121 combinations.